CLINICAL PHARMACOLOGY and THERAPEUTICS

LANE MEDICAL LIBRARY
JAN 26 1960

Official publication of the American Therapeutic Society

volume 1 *number* 1 **January-February 1960**

Appearing in this issue

Man's hand in bacterial resistance
Ernest Jawetz, M.D., San Francisco, Calif.

Introduction to Symposium on the study of drugs in man
Leroy D. Vandam, M.D., Boston, Mass.

Symposium Part 1. From animal to human
Theodore Koppanyi, Washington, D. C.

Epinephrine and norepinephrine, actions and use in man
U. S. von Euler, M.D., Stockholm, Sweden

Newer drugs in amebiasis
Hamilton H. Anderson, M.D., San Francisco, Calif.

The actions and uses of anthelmintics
Harold W. Brown, M.D., New York, N. Y.

Modern treatment of the systemic fungus diseases
E. Richard Harrell, M.D., and Florante C. Bocobo, M.D., Ann Arbor, Mich.

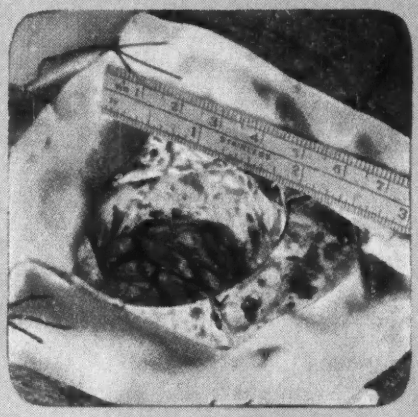
Complete Table of Contents, page 1

Published at St. Louis 3, Missouri by **The C. V. Mosby Company**

BINDERY

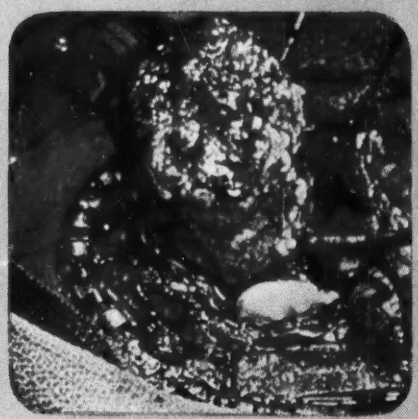
a new sound-color film...

"A new approach to the reduction of intracranial pressure with Urea-Invert sugar"



UREVERT™

(LYOPHILIZED UREA AND TRAVERT®)



The clinical data presented in the film are taken from the case histories of more than 550 patients who received urea-invert sugar solution [UREVERT™] at the University of Wisconsin hospitals for a variety of cranial disorders.

The use of Urevert to facilitate intracranial surgery in glioblastoma multiforme, optic nerve glioma, fronto-temporal meningioma, cerebellar astrocytoma and retrogasserian rhizotomy is illustrated.

A comparison of Urevert with other hypertonic solutions demonstrates its superiority to such agents in reducing cerebrospinal fluid pressure and brain volume.

For Your Group—showing of this 19 minute film may be arranged by writing to Medical Film Library, Travenol Laboratories, Inc., Morton Grove, Illinois.

TRAVENOL LABORATORIES, INC.

pharmaceutical products division of

BAXTER LABORATORIES, INC.

Morton Grove, Illinois

CLINICAL PHARMACOLOGY and THERAPEUTICS

Editor

Walter Modell, M.D.

Editorial Board

W. A. Bain, M.D., Ph.D.
Edward A. Carr, Jr., M.D.
Windsor Cutting, M.D.
Arthur C. DeGraff, M.D.
James M. Dille, M.D.
Alan K. Done, M.D.
Dale G. Friend, M.D.
George C. Griffith, M.D.
Arthur Grollman, M.D.
Harriet Hardy, M.D.
Raymond W. Houde, M.D.
Ernest Jawetz, M.D., Ph.D.
David A. Karnofsky, M.D.
Kenneth G. Kohlstaedt, M.D.
Herbert S. Kupperman, Ph.D., M.D.
D. R. Laurence, M.D.
T. A. Loomis, M.D., Ph.D.
Donald Mainland, M.B., D.Sc.
H. Houston Merritt, M.D.
Eric Nilsson, M.D.
Carl C. Pfeiffer, Ph.D., M.D.
William B. Rawls, M.D.
Marion B. Sulzberger, M.D.
Leroy D. Vandam, M.D.
Walton Van Winkle, Jr., M.D.
Andrew Wilson, M.D.
Gerhard Werner, M.D.

Volume 1, January-December, 1960

The C. V. Mosby Company, St. Louis

Volume 1
Copyright © 1960 by
The C. V. Mosby Company

All rights reserved

Printed in the United States of America

CLINICAL PHARMACOLOGY and THERAPEUTICS

volume 1 *number* 1 **January-February 1960**

Editorial

Walter Modell, M.D. *New York, New York*
Editor, Clinical Pharmacology and Therapeutics

The purpose of *Clinical Pharmacology and Therapeutics* is to bring together publications of high quality on problems in the evaluation of the actions and effects of drugs in man. At present such publications are scattered throughout the medical literature simply because there is no journal which has as its sole interest the field of human pharmacotherapeutics. To date there is no journal of clinical pharmacology, no journal of therapeutics as such. There is no frame for a fair and representative picture of this vital, prolific, aggressive, confusing, misused, hopeful, underrated, and, above all, wish-propelled field of modern medicine. There is no journal to control and curb its exuberance.

Because of the tremendous number of new drugs now being regularly introduced into medicine, problems of their pharmacologic and therapeutic evaluation have now become matters of great practical importance. As a result, clinical pharmacology, which has been some fifteen years in maturing, is now being recognized as a proper discipline in medical science.

Departments and divisions of clinical pharmacology have already been established in some medical schools, outstanding hospitals, and research institutes, and are being established in others. In England the first international symposium on methodology in human pharmacology was held in 1958.

In spite of these recent acknowledgments of an important discipline in medicine, much is still published about drugs and therapeutics in outstanding journals which is neither good nor acceptable. This is largely because most medical journals have no editorial standards for investigations of drug actions in man and their editorial boards are not properly geared to screen or edit publications resulting from them. By virtue of the specialized knowledge of its editorial board, a journal specializing in problems of drug evaluation in man would exclude poor papers. While this editorial screening by specialists in therapeutics as well as clinical pharmacology would, by itself, insure high standards of publication, the focalization of papers in the field would tend to elevate the standards of the field in general. It would tend to improve methods of investigation, to add insights into the subject, and

to lead to more incisive analysis of the data. It would not only make the comparison of one paper with another easier, it would make it unavoidable, and would thereby encourage the critical examination of methods of investigation or analysis of results.

Clinical Pharmacology and Therapeutics is meant for the therapeutist and for the clinical pharmacologist. The practice of empiric medicine is declining, while the modern practitioner is showing a growing interest in the pharmacologic basis of his therapy as a consequence of modern methods of medical education and because his practice requires that he know about the many potent pharmacotherapeutic agents constantly being introduced on the drug market.

Clinical Pharmacology and Therapeutics will provide the perceptive practitioner with up-to-date information of the type he needs and seeks, much of which takes years to find its way into standard textbooks of pharmacology and therapeutics. It will offer substantial opinions on current drugs and therapies for which he now has to wait out the far-too-long time lapse before they appear in general medical publications. The urgency of the need for prompt and unbiased information was emphasized by an outstanding medical voice which proclaimed in the newspapers recently that medical advertisements were becoming a major influence in shaping therapeutic practices.

The clinical pharmacologist and the therapeutist will find in *Clinical Pharmacology and Therapeutics* not only a place to have his contributions published in good company, but also a place where he is an insider rather than an outlander. His papers will benefit from specialized editorial advice. His files of *Clinical Pharmacology and Therapeutics* will provide him with the most likely source of information on particular drugs and methodology in the investigation of the effects of drugs in man.

The scope of the Journal is indicated by its title. It will concentrate on the *effects of drugs in man*, their proper and enduring evaluation, their actions, and their therapeutic and toxic effects. It will therefore publish original articles of high quality on clinical and therapeutic evaluations of new drugs and re-evaluations of old drugs as well. It will contain articles on the general pharmacology of drugs in man. It will publish concise review articles and commentaries on active fields in pharmacotherapeutics in an effort to bring together scattered and undigested information and to analyze the situation in an expert, but also realistic and practical way. It will devote occasional issues to symposia on currently active fields in pharmacotherapeutics. It will review books on pharmacology, therapeutics, and closely related fields in the basic and the practical aspects of clinical medicine. It will contain brief commentaries on the latest drugs and drug products introduced by pharmaceutical manfacturers, with the advice and prognostications of authorities before judgments based on the large clinical experience on the drugs are available. There will be a correspondence section for less formal statements on experiences with new drugs. Editorials commenting concisely on important aspects of pharmacotherapy will be a regular feature.

There will be no articles on animal experimentation per se; original data on animal experiments will be strictly limited to that which is an integral part of, or basic to, studies which deal primarily with the effects of drugs in man.

Man's hand in bacterial resistance

Ernest Jawetz, M.D. *San Francisco, Calif.*
Department of Microbiology, Medicine and Pediatrics, University of California Medical Center

Medical journals and the lay press have recently carried many accounts of the ravages wrought by antibiotic-resistant bacteria in outbreaks of hospital infection. The current emphasis on these problems might suggest that they are new in medical thought and experience. Nothing could be farther from the truth. Outbreaks of hospital infection presented frequent difficulties in the preantibiotic era.[1] Resistance to antimicrobial drugs was well known since Ehrlich's[2] masterful descriptions and predictions in 1913. Thus we must accept that both hospital-induced infections and bacterial resistance cannot be blamed entirely on the abuses of antimicrobial drugs and neglect of sound medical principles during the past fifteen years of the "antibiotic era." Nevertheless, it may be desirable to call attention to some of the practices and attitudes which unquestionably have contributed to the magnitude of the present difficulties.

At the outset it must be stated that the problem is too complex to permit any simple all-inclusive explanations or easy remedies. Only two of many interrelated facets are presented here: the selection pressure of antimicrobial drugs used on a large scale, and the deterioration in aseptic practices largely stemming from unreasonable confidence in antimicrobial drugs.

Fortunately a majority of disease-producing bacteria remain susceptible to several effective drugs. Only a few kinds of bacteria (e.g., Proteus, Pseudomonas) tend to be uniformly resistant to most antimicrobial agents. In some others (e.g., Aerobacter, Staphylococcus) a large portion of the bacterial population is drug susceptible but spontaneous resistant mutants emerge frequently. A significant level of antimicrobial drug in the environment affects both of these types of bacterial populations. In the first instance the drugs favor implantation or proliferation of resistant microbial species by suppressing the susceptible normal flora and thus creating a void which must be filled. In the second instance the growth of the drug-sensitive members of the microbial population is suppressed by the drug, permitting the spontaneously arising resistant mutants to proliferate without restraint. In either case the drug exerts selection pressure in favor of the resistant microorganism and disturbs natural balance.

These mechanisms may apply within a single human being or in an environment shared by many people, e.g., a hospital. In a patient suffering from a serious staphylococcal infection the microbial population may shift from apparent susceptibility to resistance during the administration of such single drugs as streptomycin, erythromycin,

or novobiocin.[3] The drug in tissues initially suppresses the bulk of the microbial population, which is susceptible, but permits the few resistant mutants to survive and multiply. After an initial favorable clinical response the disease relapses as the resistant organisms "take over." In such staphylococcal disease, as well as in tuberculosis, the emergence of drug-resistance may be delayed by the simultaneous use of two effective drugs, rather than a single one.[4]

When a given antimicrobial drug is used on a large scale in a closed environment such as a hospital, microbes resistant to that drug are favored. They prevail in the area as they establish themselves in the occupants (nurses, physicians, patients) and replace drug-susceptible microbes. This had been clearly demonstrated for erythromycin.[5] Just a few months after this drug began to be employed on a large scale the originally erythromycin-susceptible staphylococci colonizing skin and respiratory tract of patients and personnel were to a large extent replaced by erythromycin-resistant ones. A similar train of events has occurred with other drugs, and is likely to follow the introduction and large-scale use of *any* new drug. Only by a drastic reduction in the over-all use of antibiotics and restrictions placed on specific new drugs[6] can we hope to diminish the magnitude of these difficulties. Once drug-resistant microorganisms prevail in a hospital the removal of the offending drug can reduce, but cannot eliminate, the resistant mutants. The emergence of drug resistance in hospitals depends in part on the selection pressure of drugs given to patients, in part on the intensive exchange of organisms in the closed environment, and in part on the direct effect of drugs in the atmosphere, as demonstrated for penicillin by Gould.[7] The implantation into patients of prevailing drug-resistant bacteria is enhanced by suppression of their normal microbial flora by antibiotics. Knight and associates[8] have shown that patients receiving tetracycline or penicillin acquire drug-resistant staphylococci much sooner after admission to the hospital ward than patients not receiving those drugs. This evidence should persuade the physician to prescribe antibiotics only for specific indications and not for vague and irrational "prophylaxis."[9]

While the abuse of antibiotics plays an important role in selecting out drug resistant microorganisms and favoring their survival, much of the dissemination of these resistant organisms must be blamed on a loss of respect for aseptic techniques. With the advent of powerful antimicrobial drugs, some fifteen years ago, the carefully established methods for the restraint of communicable diseases were largely abandoned. Physicians believed that antibiotics would not only cure all infectious diseases but would also eliminate all microbial pathogens from the earth. Thus there seemed to be no further need to observe aseptic or isolation precautions. Handwashing became a lost art and remained so until the recent upsurge of infections in nursery and surgical patients. At present respect for cleanliness and asepsis is slowly returning. But even now the patient discharging a vast number of drug-resistant staphylococci is not considered as dangerous as the person discharging typhoid bacilli, and isolation is rarely enforced. The high density of drug-resistant bacteria in our hospitals has resulted in high carrier rates in hospital personnel, particularly in physicians and nurses, and such individuals contribute to the reservoir of deadly organisms in our modern "pesthouses." Newborn infants all too often acquire drug-resistant bacteria in the nursery and carry them home to their siblings and parents. In this fashion the family becomes a reservoir of drug-resistant bacterial infection.[10] Penicillin-resistant staphylococci are now found in about 45 per cent of children under 5 years of age.[11] Adults in the community also are acquiring drug-resistant bacteria at an increasing rate. Among outpatients with hand infections in one British infirmary no penicillin-resistant staphylococci were found in 1952 but were found in 30 per cent in 1957.[12] The end of the problem is clearly not in sight. How shall we counteract the trend?

One easy solution would be the frequent advent of new and better drugs. The experience of the past few years offers little encouragement. New drugs (not reformulations of old ones) have been few and have compared unfavorably with earlier ones in regard to efficacy and toxicity. Thus we are forced, with regret, to rely on conserving the older, established antimicrobial agents by drastically reducing their abuse. Renewed emphasis has to be placed on asepsis, isolation, and cleanliness as a means of reducing the spread of drug-resistant organisms. Research is needed into the nature of bacterial virulence and persistence and the characteristics of the human carrier state, and its control. It is disturbing to note that occasionally even well-conceived and adequately maintained efforts to deal with a high level of drug-resistant infection end in failure.[13] When contemplating the future role of "man's hand in bacterial resistance" one must hope that it will be cautious—and well scrubbed.

References

1. Devenish, E. A., and Miles, A. A.: Control of Staphylococcus aureus in Operating Theatre, Lancet **1**:1088-1094, 1939.
2. Ehrlich, P.: Chemotherapeutics: Scientific Principles, Methods and Results, Lancet **2**:445-451, 1913.
3. Finland, M.: Emergence of Antibiotic-Resistant Bacteria, New England J. Med. **253**:909-922, 1955.
4. Jawetz, E.: Combined Antibiotic Therapy in Serious Infections Caused by Drug-Resistant Staphylococci (1956-1957), A.M.A. Arch. Int. Med. **103**:289-307, 1959.
5. Lepper, M. H., Dowling, H. F., Jackson, G. G., Moulton, B., and Spies, H. W.: Effect of Antibiotic Usage in the Hospital on the Incidence of Antibiotic-Resistant Strains Among Personnel Carrying Staphylococci, J. Lab. & Clin. Med. **42**:832, 1953.
6. Jawetz, E.: Patient, Doctor, Drug and Bug (The Randall Lecture), New England J. Med. **258**:785-791, 1958.
7. Gould, J. C.: Environmental Penicillin and Penicillin-Resistant Staph. aureus, Lancet **1**:489-493, 1958.
8. Knight, V., White, A., Foster, F., and Wenzel, T.: Studies on Staphylococci From Hospital Patients. II. Effect of Antimicrobial Therapy and Hospitalization on Carrier Rates, Ann. New York Acad. Sc. **65**:206-221, 1956.
9. Howe, C. W.: Postoperative Wound Infections Due to Staphylococcus aureus, New England J. Med. **251**:411-416, 1954.
10. Kempe, C. H.: The Family as a Reservoir of Childhood Infection, J.A.M.A. **151**:1472-1474, 1953.
11. Hurst, V.: Penicillin Resistance of Staphylococci Carried by Infants and Young Children, J. Lab. & Clin. Med. **52**:254-258, 1958.
12. Buhr, A. J., and Scott, J. C.: Penicillin-Resistant Staphylococci Incidence in Outpatients With Hand Infection, Lancet **1**:1019-1021, 1959.
13. Williams, J. R. B., Talbot, E. C. S., and Maughan, E.: Hospital Outbreak of Cross-Infection Due to Staphylococcus pyogenes, Phage Type 80, Brit. M. J. **1**:1374-1378, 1959.

Symposium on the study of drugs in man

Introduction

The creation of a new journal devoted to clinical pharmacology and therapeutics is recognition of a relatively recent phenomenon in medicine, that is, the strenuous effort to develop new drugs to influence favorably the course of disease. In the preparation of these drugs there has been the understanding by pharmacists, pharmacologists, and clinicians alike that the final answer to the efficacy of these drugs can be had only after trial in man. Thus the physician has been besieged in recent years with a spate of articles describing the clinical effects of newly synthesized drugs. These articles are often difficult to evaluate, are not infrequently repudiated, and reports of dangerous side reactions sometimes follow in their wake. The underlying reason for these problems probably lies in the complexity of studies made in man. Not only is this a new field but a host of problems of a scientific, moral, and legal nature are brought to the fore.

With a realization of the aforementioned facts the editors of CLINICAL PHARMACOLOGY AND THERAPEUTICS have arranged a symposium on "The Study of Drugs in Man." The term "human experimentation" has been deliberately avoided to escape the common connotation or association that experimentation in man is allied to malpractice. The contributors to this symposium are outstanding men in their respective fields. Five articles will appear in successive issues of this journal. The first is by Dr. Theodore Koppanyi, Professor and Chairman of the Department of Pharmacology at Georgetown University School of Medicine. Dr. Koppanyi is a classical pharmacologist who has made and participated in many studies of drugs in man. Subsequent contributions to this colloquim will be: "Biologic and Medical Studies in Human Volunteer Subjects," by Francis D. Moore, M.D., Moseley Professor of Surgery at the Harvard Medical School and Surgeon-in-Chief at the Peter Bent Brigham Hospital, Boston; "The Moral Aspects of Human Experimentation in Medicine," by John J. Lynch, S.J., Professor of Moral Theology at Weston College in Massachusetts; "The Ethics of Medical Research," by Seymour J. Kety, M.D., Chief of the Division of Neurological Diseases and Blindness, at the National Institutes of Health; and "The Legal Considerations of Medical Experimentation," by Irving Ladimer, S.J.D., formerly Assistant Chief of Research and Planning at the National Institutes of Health and United States Public Health Service.

Leroy D. Vandam, M.D.

Part 1. From animal to human

Some basic principles of comparative pharmacology

Theodore Koppanyi *Washington, D. C.*
Department of Pharmacology, Georgetown University School of Medicine and Dentistry

Clinical pharmacology, to which this JOURNAL is devoted, is rapidly taking its place in the ranks of exact sciences. Neither clinical pharmacology nor its derivative, rational therapeutics, is a lineal descendant of the empirical treatment of disease.[1] They are built on the basis of the relatively new science of comparative pharmacology, which ascertains the obvious, and, with improved techniques, the less obvious effects and probable dangers of drugs.[2] For example, digitalis has been used empirically for a long time in the treatment of edema, but it was only after the lapse of a century that it became known as a cardiac stimulant, producing diuresis through its effect on the myocardium. Today it would be anachronistic to use digitalis glycosides in edema of other than cardiac origin. Clinical pharmacology and rational therapeutics are based upon the understanding of drug action and not upon the empirical principle of trial and error.

I. Animal and human (clinical) pharmacology

A. Scope. Pharmacology is a science of natural phenomena dealing principally with the measurable, predictable, and therefore reproducible effects of drugs on function and on the cellular structure of animals and humans. Although it employs no methodology or technology of its own and borrows its procedures from chemistry and physiology (biochemistry and biophysics), this does not minimize its fundamental importance.

The significance of *autopharmacology* versus *heteropharmacology* stems from the fact that pharmacology deals not only with biologically occurring chemicals with specific actions but also with drugs which, by definition, are administered from without. Very often this distinction is not sharp, since many biologically occurring substances can be synthesized and administered as drugs. These naturally occurring chemicals (amino acids, nucleotides, nucleosides, hormones, neurohormones; or vitamins as cofactors) are mostly true metabolites essential for vital processes. Inhibition of specific cellular functions occurs when such metabolites are replaced by exogenous chemicals which compete with essential biogenic substances because of similar chemical structure or for some unknown reason. Such competing drugs are antimetabolites.[3] Several antimetabolites, such as sulfonamides, are now routinely used with safety in clinical medicine.[4]

Animal pharmacology for practical purposes may be subdivided into fundamental pharmacology and the pharmacology of screening new drugs.

The task of fundamental pharmacology is the investigation of biologic phenomena with the aid of established or new drugs as tools and the study of the effects of drugs and their natural or synthetic analogs. In this case the action of the drug is of primary interest.

Pharmacology of screening new drugs is almost feverishly engaged in testing an amazing number of synthetic chemicals on cells, tissues, organs, or the whole animal. Acute, subacute, and occasionally chronic toxicities are also determined.

In both types of pharmacology, structure-activity relationships (SAR) are becoming increasingly important.

Human pharmacology is the study of drugs in the genus Homo including the normal human being as well as ambulatory or hospitalized patients.

It is necessary for the pharmacologist and clinician to establish whether or not the pharmacologic effects in humans and animals are identical (animal hides respond to drugs differently from human skin). False positive or false negative reactions can occur which make the task of the pharmacologist and clinician more difficult. A false negative test is one in which a drug produces a desired effect in humans but fails to produce a similar effect in normal animals (nalorphine is a potent analgesic in humans but not in animals). A false positive test is one in which a drug produces a distinct pharmacologic effect in animals but fails to produce similar effects in the human patient (vasopressor drugs for animals are usually not useful in human shock). Because of the existence of "false negatives" many useful drugs may be overlooked and never reach the stage of clinical trial. On the other hand, many drugs highly effective in animals may cause a great deal of wasted time and resources in clinical trials. It should be emphasized that these statements about "false negatives" and "false positives" are only heuristic concepts. They represent judgments of value and purely practical considerations without affecting the fundamental task and goals of animal and human pharmacology.

Not only may the drug actions in humans deviate from those observed in animals, but almost always the quanta necessary to elicit pharmacologic responses differ from species to species.

Another reason why drug actions in man may differ from those seen in animals is that most animal experiments are carried out in spinal preparation or in animals under various types of anesthesia. These preparations do not represent the complete counterpart of the intact, unanesthetized human organism.

In investigating a drug in humans, one should be sufficiently sophisticated to test at first the effects of the single drug and make certain that combined drug actions in the form of synergisms or antagonisms do not obscure the true pharmacologic effect of the drug under investigation.

Human pharmacology must also concern itself with the frequency of side effects of drugs. For example, an analgesic which causes vomiting at the optimum point of analgesia is not a useful agent.

Toxic effects of drugs need not be identical in animals and humans. For example, an otherwise satisfactory tranquilizer may not cause optic damage in animals, but it certainly produced retinitis pigmentosa in humans. Therefore, an adequate number of patients should be tested intensively to unmask undesirable and toxic effects of drugs before they are released for routine clinical use.

Clinical pharmacology is really a team effort. In contrast to animal pharmacology, where an assumption is being put to an experimental test, the clinical pharmacologist deals with a drug which has already been evaluated in animals. Here, success depends upon the services of three scientists—a clinician, a pharmacologist, and a statistician. Although their task calls for a "fusion of talents" they also have individualized functions to perform. The clinician is responsible for setting up the criteria for the trial and devising methods for the collection of critical data. No one can quarrel with Lasagna[5,6] that the clinician alone has the all important background for the clinical trial, the knowledge of the nature, course, and prognosis of the disease—in short, the necessary clinical experience. The pharmacologist contributes the already existing knowledge about the drug to be tested, so

that "rational dosage schedules can be set up."[5] The statistician guards against bias in planning the experiment. Therefore, he should not be called in at the conclusion of the experiment as an afterthought,[7] but should be consulted at the beginning to participate in formulating the experimental procedure, since the experimental plan and design are just as important from a statistical viewpoint as the final analysis.

B. Historical retrospect. It appears that the scientific study of drug action was a direct consequence of Harvey's discovery of the circulation of the blood. It originated in England and Switzerland. It is almost certain that the first suggestion to investigate drug action in animals by the intravenous route came from the famous London architect and amateur scientist, Sir Christopher Wren. Apparently at his suggestion, Robert Boyle (1660) administered a number of crude drugs intravenously to dogs, and measured their effects. He showed that opium and antimony, when taken by mouth, act after absorption into the blood stream. He may be considered as the founder of experimental pharmacology. John Jacob Wepfer (1679) of Schaffhausen, Switzerland, discovered drug-induced convulsions, and endeavored to determine the acute toxicity of a number of drugs used in his time. On the basis of his toxicologic studies, he protested against the indiscriminate use of mercury and arsenic.

In 1776, a young German pharmacist's apprentice, Peter John Andrew Daries, published a doctor's thesis, "On Atropa Belladonna," which is perhaps the most important pharmacologic contribution of the eighteenth century. Daries, in carefully controlled experiments on himself and in cats, discovered the mydriatic effect of belladonna preparations, and made the fundamental cautious generalization that the effect of drugs varies directly with the size of the dose.

The physiologic methods essential for the birth of modern pharmacology were introduced almost simultaneously with the isolation of pure drugs. It is fortunate that the great French physiologists in the first part of the nineteenth century (Magendie, Flourens, and Bernard) used drugs in their physiologic researches, and thus opened the way for the systematic study of drug actions.

Pharmacology as an independent discipline owes its origin to German scholars, Buchheim, Schmiedeberg, and Binz. Somewhat later, British and American pharmacologists helped to develop this new scientific discipline.[8]

Human (clinical) pharmacology, while in a sense as old as medicine, did not seem to achieve the status of a critical experimental discipline until the discovery by James Lind (1747) of the curative effect of oranges and lemons in scurvy.[9] It is only recently that human pharmacology, satisfying the most stringent criteria, came into being. Only in 1952 was clinical pharmacology actually defined as a body of knowledge by Drs. Harry Gold in this country and J. H. Gaddum in England.[9,10] According to these authors, clinical pharmacology consists of the application of pharmacologic tests to obtain objective evidence of the value of drugs in patients.

C. Objectives of animal and human pharmacology. The main objective of *animal pharmacology* is to study drugs and to determine their specific effects, sites of action, and mechanisms of action by a variety of objective criteria, including adequate control experiments. An "inactive" placebo, such as physiologic salt solution, or a "standard reference," such as morphine when a new narcotic analgesic is tested, will be used according to the problem under investigation. In addition, the pharmacologist must accumulate pertinent data about drugs given by various routes, elucidating the onset and duration of action, the metabolism and elimination, and dose-response relationships.

If, in the mind of the investigator, the drug might be useful in therapy, then acute, subacute, and possibly chronic toxicity experiments should be performed. Special attention is paid to the effects on the

central nervous system, bone marrow, kidney, and liver. The incidence of side effects such as anorexia, nausea, vomiting, diarrhea, etc., is noted and evaluated. Toxicity studies should be terminated at different time intervals, and macroscopic and microscopic examinations of organs performed. Chronic toxicity tests are necessary only for drugs that are to be taken for years and for all food additives. If, for example, the value of digitalis had been only recently discovered, chronic toxicity studies would be imperative. As it is, because chronic toxicity tests were not required at the time digitalis was introduced, they were not performed and the "hormonal" activity (e.g., gynecomastia) had been entirely overlooked, and was disclosed only recently by clinical observation.[11]

In many pharmacologic experiments, animal behavior must be of central interest to obtain a complete picture of drug action. This is especially true of the psychopharmacologic agents. Whenever possible, investigators in the field of animal psychology should be consulted in these studies in the same manner as clinical psychologists are consulted in the clinical trial period of similar drugs. The influence of such drugs on the speed of the learning process, for instance, can be better evaluated by a psychologist who is an expert on animal behavior.

In *human pharmacology,* we assume that the principal pharmacologic and toxicologic effects, including the site and mechanism of drug action, as well as the fate of the drug, have already been ascertained. The gross pharmacologic effects may be similar, but there is often a difference in sensitivity, with humans being either more or less sensitive than many laboratory animals.

Human pharmacology at the present time is concerned with the gross effects of drugs in contrast to animal pharmacology which additionally concerns itself with the drug effects on the cellular and even subcellular levels. But since newer methods in clinical medicine have been developed, such as liver and renal biopsy and removal of portions of the central nervous system, human pharmacology may well include such topics as drug action on enzyme systems (liver microsome, mitochondria preparations) among others.

To discover which effects of drugs are different in the human species from the effects in other animal species is an objective of human experimentation. Human volunteers or patients may be used for such experimentation. It has become increasingly common to use human volunteers, both paid and unpaid. The data obtained from volunteers are useful as a base line against which later studies in patient groups are conducted.[12] Experimental results obtained by the use of volunteers as subjects should be reviewed with caution, however. One must be constantly aware that volunteers in an experimental situation are "different" as a group from those less eager to participate in a clinical trial. Numerous psychological measurements have revealed significant differences in this population when compared with the "normal" population group.[12]

However, the "psychological" effects of drugs can usually be distinguished from the primary drug effects, if the drug is given in supratherapeutic doses.[13] In the absence of a supratherapeutic dose schedule, placebos are essential, even though a large number of placebo reactors are usually encountered in a clinical experiment. If the placebo reactors are eliminated and the experiment repeated (testing once more an active ingredient versus placebo) the results thus obtained will be different and often no effect will be observed following placebo medication.[5,14-16]

The dose-response curve for drugs should be established. The dose of any drug in humans is essentially the same—that is, enough. The dosage, then, is the amount of drug needed to produce the optimum pharmacologic or therapeutic effects. If minor toxic symptoms should develop before the onset of optimum desired effects, discontinuation of the drug may be indicated. It is therefore imperative in human pharmacology to start with a dose which may seem

disproportionately low when compared with effective doses in animals.[1] The dose is then gradually increased until optimum effects are obtained. If this procedure had been followed meticulously in the past, we would not now be confronted with the anomalous situation represented by the usual dose of morphine, which is twice as great as is needed for severe pain. This dose-response curve in humans should be determined as part of the pilot study before the drug is submitted to a large scale clinical trial.

II. Experimental design

A. Pilot study. Original hypotheses concerning drug action should be tested on a few animals to obtain experimental confirmation. This is called the preliminary or pilot study. The experimental results obtained will indicate only a trend. To interpret the trend we run the risk of committing two types of errors. In errors of Type I the investigator may overlook the trend and this may lead him to reject the hypothesis when it should be accepted. In errors of Type II the investigator accepts a hypothesis which should be rejected.[17] Therefore, if the initial testing is confirmatory and the level of significance has been agreed upon, it is imperative to initiate a well-controlled experiment designed to re-evaluate the preliminary study. Only too often a trend is mistaken for a complete investigation, or the experiments stop at the pilot level. This serves only to aid in cluttering the literature with possibly misleading material. It is because of the mistaken identification of a pilot study for a more definitive study that the original investigator or someone later repeating his experiments is frequently biased.

B. Complete study. When the study is beyond the pilot stage, then a statistical blueprint of the experimental design is necessary to keep the experiment free from bias or systematic errors of allocation or assessment. In a full-scale study, it is the statistician or the statistically trained scientist who has the last word about experimental design. He can be the most critical of how close the contemplated plans will come to the objectives of the study. For example, if bone marrow depression occurs in 0.5 per cent of experimental animals receiving a particular drug, it is obvious to the experienced investigator that a study of 75 or 100 patients might not reveal any instances of bone marrow depression. This would result in a false sense of security as to the safety of the drug.

C. Randomization. Random sampling is one way of insuring the validity of selection of a fair sample of the population under investigation and may be procured by various artificial means. It is diametrically opposed to purposive sampling, where the investigator selects his sample not arbitrarily (e.g., elimination of placebo reactors) but with as many common denominators as possible. Randomization carries with it the uncertainty of chance fluctuation, whereas purposive sampling may be influenced by the bias of the investigator. It is necessary to avoid bias in the allocation of patients or animals to different treatment groups. The way in which the groups are picked is not important for random sampling, provided that safeguards are taken to insure that the groups are actually selected without premeditation. For example, it would usually not matter if one were testing the use of Drug A in the treatment of motion sickness if every other passenger were given the drug (provided this does not lead to "chance selection"), or if the choice were made by spinning a wheel with black and red marks. The passenger allotted a red mark would receive the drug, and the passenger allotted a black mark would get the placebo. It is, of course, important that the number of drug-treated and the number of placebo-treated cases be approximately equal. If such a simple method of random sampling were used, the group receiving the placebo aboard ship would not be exposed predominantly to one set of spatial and environmental conditions, and the passengers receiving the drug to another.

The same principle of randomization applies to the use of hospital patients whether or not the study is conducted in a single hospital or simultaneously in several hospitals. It is needless to add that the same principle should be applied to animal experimentation in a somewhat modified form.

Another way of setting up adequately controlled experiments is to let the animal or patient serve as his own control—i.e., by random administration of drugs and control preparations in the same test subject without predetermination ("crossover" experiments).

D. "Double blind" tests or "unknowns technique." In the double blind test, the patient and the person observing the drug effects are "blind," i.e., neither knows what medication has been taken. The purpose of the double blind study is to eliminate the enthusiasm of the observer and the patient, which might affect subjective responses or even objective signs (a placebo may increase the frequency of micturition and total urine output, or can act as a cathartic). As far as I could ascertain the double blind test was first described and used by Gold, Kwit, and Otto.[19] In their study of xanthines on cardiac pain they mention that "in a further attempt to eliminate bias, the questioner usually refrained from informing himself as to the agent that had been issued until after the patient's appraisal of the period had been obtained."

The human species is unique in that it is the only form of life that can communicate with the observers, and make recorded observations on himself. It is also the only species that is open to suggestion. Therefore, man is the only organism in which the double blind test, or as Beecher terms it, "unknowns technique," is necessary.[14]

The simplest method for evaluating a drug is to study its effects against a passive placebo or active placebo or a reference standard ("yardstick placebo") or any combination of the three. The ideal active placebo would have the same side effects as the drug, without its therapeutic action, a rare combination which is difficult to supply.

Errors in the double blind test frequently are centered around the use of placebos. If a drug being tested has side effects which are easily experienced by the subject, it is obvious that a passive placebo will be recognized. It is equally obvious, then, that a placebo should not be differentiated easily from the substance being studied in order for the experiment to be truly double blind. It is therefore often better to employ in addition to the passive placebo an active placebo, or if this cannot be supplied, a reference standard.[5]

Recently a new addition to the design of the double blind or "unknowns technique" method has been presented by Fazekas and associates.[20] These authors are comparing the effect of anorexigenic drugs on weight loss in mentally alert and in mentally deficient subjects with a mean I.Q. of about 37. It is their hope that, in the mentally deficient subjects, the placebo reaction as well as the whole emotional reaction toward the drug administration will be minimized, and obviate, in so far as possible, the power of suggestion.

The double blind test as a clinical experimental design has not gone unchallenged. Batterman and Grossman[21] claim that the double blind technique as applied to analgesic and antirheumatic drugs in ambulatory patients does not show any significant differences between placebo therapy and the drug involved in the case, mainly aspirin. They found the same effectiveness for placebos, "known effective analgesics (aspirin and N-acetyl-aminophenol) and unknown drugs." It is probable that their criticism has already been answered by Modell and Houde[27] and also by Beecher and co-workers[22] who found no appreciable differences in effectiveness of analgesics and antirheumatic drugs tested in placebo-reacting patients, but highly significant differences in nonreactors.

E. Paired controls. These controls are usually paired with respect to one common denominator, e.g., food intake. In

studying the nutritional requirements of tumor-bearing animals, one animal with neoplasm is allowed to feed ad libitum, while a non-tumor-bearing mate is fed an amount of food equivalent to that consumed by the test animal. In this manner the total caloric intakes of both animals are made equivalent as nearly as possible. Thus the effects of tumor growth are separated from those of general nutritional deficiencies.

If a drug is administered to animals or patients, while others are kept as controls in the same place and at the same time, it is possible with suitable precautions to show that the only factor which produced the observed change between two animals, two patients, or two groups of animals or patients must be due to the drug. We call this method simultaneous controls.[9]

F. Significance of results. The significance of the experimental results depends upon their appraisal and summary. Statistical analysis which accomplishes these ends is particularly desirable in animal and human pharmacology because of the variability of results caused by unknown or difficultly controllable factors. Statistical methods enable the investigator to eliminate the variables that may interfere with the interpretation of the results, and they should also furnish a clue to cautious generalizations drawn from the experiment, i.e., limitation of prediction.

Of course, statistical methods cannot convert a badly designed experiment into a good one. As already pointed out, statistics should not only be used after all of the results are in, but from the very beginning so that statistical methods can be applied to the data as collected.[6,7]

G. Justifiable conclusions. It is customary in the pharmacologic and clinical literature to wind up the discussion of a paper with a conclusion and summary. It is of utmost importance that these conclusions should be cautious, i.e., they should emphasize that the results occur under a certain predetermined set of experimental conditions. It is of equal importance not only that the ultimate findings should be described, but that the method of sampling and other conditions under which these results were obtained be enumerated. If all the conditions under which the experiments were performed are not discussed, the experiment—no matter how revealing the results—may not be reproducible by other investigators. Among the factors that are of prime importance in altering pharmacologic responses are weight, lean body mass, age, sex, heredity, metabolic state, and, last but not least, the degree of the pathologic condition of the animal or patient. In this connection, attention must be called to the representation of the dose per unit of body weight, lean body mass, or body surface area. Pharmacologists and clinicians have made the assumption that there is a direct proportionality between dose and the above measurements. This cannot be made on an a priori basis, but has to be determined in each complete pharmacologic study and the internal evidence allowed to speak for itself.[23] Even the personality of the investigator and, of course, his method of using experimental appliances can alter pharmacologic responses. Only by the meticulous description of all these characteristics of the animal and patient population and the experimental techniques can an approximate equivalence of one experiment with another be achieved. In addition to the above, certain pitfalls inherent in animal and human pharmacologic experimentation must be avoided:

1. Lack of purity and uniformity of drugs. Chemical assays, or bioassays when the former are impossible, are needed to insure uniform responses and to detect variations in potency or contamination of different batches.[24,25]

2. Failure of intended drug administration. Precautions should be taken to safeguard each enteral or parenteral administration so that the dose that is planned is actually given. This is more easily controlled in animal experiments than in humans who may not cooperate, and who may deceive the person giving the medication. It may be that both placebos and drugs

employed during a clinical trial could be tagged with methylene blue to insure the ingestion of the tablet or capsule.

3. Failure in the prevention of interfering drug or food intake. It is just as important to guard against taking forbidden medication during a clinical trial as it is against not following a prescribed regime. Ingestion of alcohol, barbiturates, and tranquilizers may interfere with the results of the therapeutic trial. It was repeatedly observed that organic mercurials failed to produce diuresis because certain patients surreptitiously obtained salt or highly salty foods.[26]

III. Outlook for the future

Pharmacology, both animal and human, is in a state of continual growth, largely as a result of the introduction of new and highly useful drugs and the continued improvement in methodology and instrumentation.[27] A connecting link between animal and human pharmacology is the study of drug action in the experimentally produced conditions in animals which are either identical with or closely resemble the diseases for which therapeutic agents are employed in the human. It is possible that pharmacotherapeutics will achieve a status which will make many mechanical therapeutic procedures obsolete. Also, in addition to being an experimental scientist, the pharmacologist of the future may also become a theoretician. The theoretical pharmacologist will, with confidence, ask the experimental pharmacologists to test his theories. It is quite possible that the theoretician will achieve such a degree of certainty in his theories that he may entertain doubts as to the experiments if the experimental verification does not agree with his predictions. In other words, pharmacology may eventually attain the present status of physics. Pharmacology will certainly graduate from being medicinae humilis ancilla to become a truly exact science.

The author wishes to express his gratitude to his friend, Dr. Michael Corrado, whose generous assistance made the writing of this paper possible.

References

1. Koppanyi, Theodore: Applied Pharmacodynamics: Rational Therapeutics, M. Ann. District of Columbia **4**:127-132, 1935.
2. Koppanyi, Theodore: Comparative Pharmacology, Arch. int. de pharmacodyn. et de thérap. **47**:423-429, 1934.
3. Albert, A.: Selective Toxicity With Special Reference to Chemotherapy, New York, 1951, John Wiley and Sons, Inc.
4. Woolley, D. W.: The Revolution in Pharmacology, Perspectives in Biol. & Med. **1**:174-181, 1958.
5. Lasagna, Louis: The Controlled Clinical Trial: Theory and Practice, J. Chron. Dis. **1**:353-367, 1955.
6. Lasagna, Louis, and Meirer, Paul: Clinical Evaluation of Drugs, Ann. Rev. Med. **9**:347-353, 1958.
7. Hill, Bradford A.: The Clinical Trial, New England J. Med. **247**:113-119, 1952.
8. Koppanyi, Theodore: The Rise of Pharmacology, Sc. Monthly **41**:316-324, 1935.
9. Gaddum, J. H.: Clinical Pharmacology, Proc. Roy. Soc. Med. **47**:195-204, 1954.
10. Gold, Harry: "The Proper Study of Mankind Is Man," Am. J. Med. **12**:619-620, 1952.
11. LeWinn, E. B.: Gynecomastia During Digitalis Therapy, New England J. Med. **248**:316-320, 1953.
12. Lasagna, Louis, and Felsinger, John M.: The Volunteer Subject in Research, Science **120**:359-361, 1954.
13. Fazekas, Joseph H., Goldbaum, Leo R., Koppanyi, Theodore, and Shea, James G.: Study on the Effect of Overdoses of Pentylenetetrazol and Barbiturate Combinations in Human Volunteers, Am. J. M. Sc. **231**:531-541, 1956.
14. Beecher, Henry K.: Experimental Pharmacology and Measurement of Subjective Response, Science **116**:157-162, 1952.
15. Jellinek, E. M.: Clinical Trials on Comparative Effectiveness of Analgesic Drugs, Biomet. Bull. **2**:87-91, 1946.
16. Mosteller, F.: Clinical Studies of Analgesic Drugs, Biometrics **8**:220-232, 1952.
17. Dixon, W. J., and Massey, F. J., Jr.: Introduction to Statistical Analysis, ed. 2, New York, 1957, McGraw-Hill Book Company, Inc.
18. Marshall, E. K.: Clinical Therapeutic Trial of a New Drug, Bull. Johns Hopkins Hosp. **85**:221-231, 1949.
19. Gold, Harry, Kwit, N. T., and Otto, H.: The Xanthines (Theobromine and Aminophylline) in the Treatment of Cardiac Pain, J.A.M.A. **108**:2173-2179, 1937.
20. Fazekas, Joseph F., Ehrmantraut, Wilfred R., and Kleh, J.: A Study of the Effectiveness

of Certain Anorexigenic Agents, J. M. Sc. **236**:692-699, 1958.

21. Batterman, Robert C., and Grossman, Arthur J.: Effectiveness of Salicylamide as an Analgesic and Antirheumatic Agent, J.A.M.A. **159**:1619-1622, 1955.
22. Beecher, H. K., Arthur, K. S., Mosteller, F., and Lasagna, L.: The Effectiveness of Oral Analgesics (Morphine, Codeine, Acetylsalicylic Acid) and the Problem of Placebo "Reactors" and "Non-reactors," J. Pharmacol. & Exper. Therap. **109**:393-400, 1953.
23. Finney, D. J.: In Laurence, D. R., editor: Quantitative Methods in Human Pharmacology and Therapeutics, New York, 1959, Pergamon Press, Inc., pp. 52-54.
24. Wyckoff, John, Gold, Harry, and Travell, Janet G.: The Importance of Differences in Potency of Digitalis in Clinical Practice, Am. Heart J. **5**:401-412, 1930.
25. Gold, Harry, Cattell, McKeen, Kwit, Nathaniel, and Kramer, Milton: The Relative Activity of Digitalis Preparations in the Frog, the Cat and Man, and Its Bearing on the Problem of Bio-assay and So-called Deterioration, J. Pharmacol. & Exper. Therap. **73**:212-228, 1941.
26. Gold, Harry: Experiences in Human Pharmacology, in Laurence, D. R., editor: Quantitative Methods in Human Pharmacology and Therapeutics, New York, 1959, Pergamon Press, Inc., pp. 40-54.
27. Modell, W., and Houde, R. W.: Factors Influencing Clinical Evaluation of Drugs, J.A.M.A. **167**:2190-2199, 1958.

(To be continued)

The uptake of thiopental by body tissues and its relation to the duration of narcosis

A mathematical analysis of the factors affecting thiopental redistribution in the human body after a single intravenous injection of the drug has been devised and presented. Concentration ratios of thiopental predicted mathematically were compared with those obtained by the direct measurement of concentrations in human blood and fat. Real and theoretical results agreed closely. According to the mathematical analysis, and also according to measurements made in man, the central nervous system attained a peak concentration of thiopental slightly less than 1 minute after intravenous injection of the drug. Five minutes later only half this concentration was present, and 10 to 20 minutes after this, only one tenth remained. The rate at which fat concentrated thiopental was too slow to explain the rate at which the central nervous system was depleted. Instead, the lean body tissues rapidly took up most of the thiopental which the brain lost. The lean tissues thus appeared primarily responsible for depleting the central nervous system of the anesthetic. These findings suggest that fat plays a smaller role in limiting the duration of thiopental narcosis than has been supposed; conversely, the mass of the body and the rate at which its tissues are perfused with blood appear more important than has been recognized.

H. L. Price, M.D.,* P. J. Kovnat, B.S., J. N. Safer, B.S., E. H. Conner, M.D., and M. L. Price, A.B.** *Philadelphia, Pa.*
Department of Anesthesiology, Hospital of the University of Pennsylvania

It is commonly believed that the anesthetic actions of thiopental are terminated, not by metabolism of the drug, but by its concentration in body fat.[2] Thiopental† is highly fat soluble,[3] but it has not been shown that its uptake by adipose tissue occurs rapidly enough to account for the speed with which consciousness is regained after intravenous injection of the drug. We have attempted to study this point.[9] Our conclusion is that fat does not play the role which has been ascribed to it. Fat concentrates thiopental so slowly that the rate of recovery from anesthesia ordinarily depends to a far greater extent upon the mass of the body than upon its fat content. Reasons for these statements, together with a quantita-

This study was supported (in part) by a grant (H-1568 C_4) from the United States Public Health Service. The computation was supported by the University of Pennsylvania Computer Center and the National Science Foundation.

Received for publication May 22, 1959.

*Wellcome Associate Professor of Research in Anesthesiology.

**Presently at University of Louisville School of Medicine, Louisville, Ky.

†Pentothal Sodium.

tive analysis of the kinetics of thiopental redistribution in the body, will be presented below.

Methods

The data reported were acquired: (1) by the direct measurement of thiopental concentration in human tissues at intervals after its intravenous injection, and (2) by construction of a hypothetical model which was analyzed mathematically, as is described in Section II.

The direct measurements were made in 8 human subjects undergoing surgical operations for the correction of varicose veins or of inguinal hernias and in 6 other subjects studied preoperatively. The first group of individuals received 0.4 mg. atropine sulfate and 100 mg. secobarbital sodium intramuscularly 1 hour prior to operation. Preliminary dissections were made with the use of local (1 per cent procaine) anesthesia; surgical repair was completed under thiopental anesthesia. Samples of blood were withdrawn from a brachial artery and fat was excised from subcutaneous tissue or omentum before and at various times after the injection of a single dose (150 to 300 mg.) of thiopental. Samples of rectus muscle were also obtained in 2 cases. Members of the second group were anesthetized with small doses of thiopental (50 to 100 mg. repeated once or twice) while sampling needles were inserted into a carotid artery and a jugular bulb under local (1 per cent procaine) anesthesia. Blood samples were withdrawn simultaneously from these vessels at various intervals after the intravenous (antecubital) injection of thiopental (150 to 350 mg.). In some cases cerebral blood flow was estimated by the nitrous oxide method of Kety and Schmidt.[6] Analyses for thiopental were performed by the method of Brodie and coworkers.[3] During anesthesia the subjects' respirations were assisted, with the use of intermittent positive airway pressure, as required to maintain respiratory minute volume within normal limits.

Results and discussion

I. Studies in man. The quantity of thiopental in human brain at various times after its intravenous injection was estimated by measuring simultaneously the cerebral blood flow and the concentrations of thiopental in carotid arterial and internal jugular venous blood. The quantity of thiopental in the brain was calculated as the integral of the arteriovenous concentration difference multiplied by the flow rate.

These studies indicated, with great consistency, that the brain rapidly took up thiopental and attained a peak content equal to about 10 per cent of the dose slightly less than 1 minute after the end of the injection. Following this, the brain lost thiopental at such a rate that only half the peak content remained 5 minutes later; at the end of 20 minutes roughly one tenth remained.

The fate of the thiopental which the central nervous system gave up was sought. According to Brodie,[3] metabolic degradation or urinary excretion could not account for such a rapid elimination of thiopental from the brain. Accordingly, the drug lost from the brain must have accumulated in other tissues.

Fat, obtained as described in the methods, was found to concentrate thiopental to only a small extent during the period considered. The highest concentrations measured during the first half hour after injection were only 1.4 times those measured simultaneously in blood. In this respect, fat behaved like other body tissues during the period considered. Since fat constitutes only one sixth or one seventh of the body mass,[7] this observation makes it unlikely that the uptake of thiopental by fat contributed to an important extent to the process responsible for depleting the brain of thiopental.

Additional measurements supported the possibility that the more slowly perfused aqueous tissues (e.g., muscle, connective tissue, bone, lung, skin) could and did deplete the brain of thiopental in the time considered. These tissues comprise roughly three fourths of the mass of the body, but receive only one fourth of its blood flow.

For this reason, they would necessarily take up their "share" of the injected thiopental comparatively slowly, and in so doing would act to deplete the brain of this drug. By "share" is meant the content in any area at equilibrium. Rapidly perfused tissues initially get more than their "share" of thiopental, and poorly perfused areas get less. As equilibrium approaches, the poorly perfused tissues therefore deplete the rapidly perfused ones of the drug. Evidence for this theory was obtained by measuring thiopental concentrations in arterial blood.

The extent of equilibration between blood and lean body tissues was estimated by employing thiopental itself as a "tag" for estimating its bodily distribution. The volume in which a substance is evenly distributed equals, by definition, the quantity of substance present divided by its concentration in that volume. Thiopental is not evenly distributed in the human body at equilibrium, but the concentrations present in blood and tissues are known with reasonable accuracy.* With the use of these data, it was estimated that the calculated volume of distribution for thiopental (dose/concentration in blood) would exceed the body volume (assuming density equal to one) by at least 20 per cent if only the watery tissues of the body were in equilibrium with the blood. This assumes no concentration of thiopental by fat. If the fat were also saturated, the distribution volume would attain 3 times the body volume. Despite this, the calculated volume of distribution failed even to equal the body volume for 15 minutes in any subject studied, and for as long as 25 minutes after the injection of thiopental in half the cases. This finding can mean only that the lean body tissues still were taking up thiopental from the blood at these times, and thus that they were depleting the rapidly perfused tissues (including brain) which had initially taken more than their "share" of thiopental. Loss of the drug from the body, if it occurred, would not vitiate this conclusion, since this would cause an overestimate rather than an underestimate of the volume of distribution.

The only lean body tissue available to us for analysis was muscle. Samples of rectus muscle obtained at herniorrhaphy in 2 cases contained less thiopental per gram than the blood did up to as long as 15 or 20 minutes after injection of the drug. Tissue/blood concentration ratios began at nearly zero and increased toward unity during the period from 1 to 30 minutes after injection.

These findings show that lean tissues can, but fat cannot, equilibrate with thiopental rapidly enough to account for the rate at which it is removed from the brain during the period considered. Unfortunately, most of the tissues which appear active in depleting the brain of thiopental are difficult to obtain in living subjects. The distribution of thiopental in the human body, therefore, cannot be measured except at autopsy. In lieu of measurement, the quantity of this drug in various areas of the body can be predicted mathematically. This approach to the problem is offered in an effort to clarify the physical factors which are responsible for the distribution of thiopental in the body at various times after the intravenous injection.

II. Construction of a model to predict thiopental distribution in the human body. In normal individuals, substances injected intravenously are rapidly delivered to and completely mixed with blood contained in a central pool of about 1.5 L. comprising the contents of the great vessels, cardiac chambers, and pulmonary vasculature. In the following analysis it is supposed that when thiopental is injected intravenously, it is mixed at once with the central pool and distributed immediately to the tissues. Since the circulation time is short in relation to the time needed for tissue equilibration, we have neglected the time needed for movement of thiopental from the heart to the tissue capillaries. It is further assumed that the blood (or lymph) leaving any tissue is in equilibrium with that tissue with

*Rieders, F.: Personal communications (based on analysis of human autopsy material).

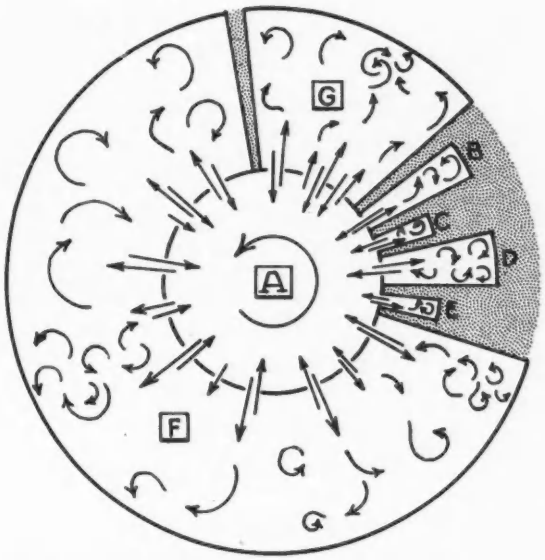


Fig. 1. Schematic diagram of the model. *A*, Pump (central blood pool). *B-G*, Various bodily tissues (compare Table I).

respect to its thiopental concentration. It is assumed that neither metabolism nor excretion of thiopental occurs during the period considered. Finally, it is assumed that thiopental injection does not alter tissue blood flow to any large extent. Reasons exist for believing that these assumptions are sufficiently correct for the present purpose.

This analysis, in effect, considers the tissues of the body as an infinite number of separate compartments which are connected by a circulatory pump. Fig. 1 shows this schematically. In order to simplify the analysis we have lumped together compartments which have similar rates of perfusion per unit mass and similar affinities for thiopental. Six such compartments are described*,[1-10] in Table I.

A mathematical solution for thiopental distribution can be written as follows:

1) Amount of thiopental injected = $M_A C_A + R_B M_B C_B + \cdots + R_G M_G C_G$

2) $R_B M_B \frac{dC_B}{dt} = Q_B (C_A - C_B)$

3) $R_C M_C \frac{dC_C}{dt} = Q_C (C_A - C_C)$

4)-7) Similarly, for D to G.

*Rieders, F.: Personal communications (based on analysis of human autopsy material).

Where C equals the concentration (w/w) of thiopental at any time in blood or lymph leaving any area (A to G), Q is the rate of blood and lymph flow through these areas, M is their mass, and R is the concentration ratio (tissue/blood) of thiopental in these areas at equilibrium. Since the equations are simultaneous, they can be solved to yield estimates of the concentrations of thiopental in various tissues at any time after its injection.* Since the characteristics of the visceral organs were similar, they were treated together as a single compartment in most solutions.

Table I. *Characteristics of human body compartments*

	Compartment	*Blood flow (ml./min.)*	*Mass (Kg.)*	*Ratio of equilibrium concentrations of thiopental (tissue/blood)*
A	Central blood pool	5,400	1.5	1.0
B	Central nervous system	800	1.3	1.4
C	Myocardium	250	0.3	1.2
D	Portal circulation	1,700	2.5	1.9
E	Renal	1,000	0.3	2.0
	Visceral	3,750	4.4	1.5
	Visceral	3,750	4.4	1.5
F	Other aqueous tissue	1,400	55.0	1.5
G	Fat	250	10.0	11.0
	Total	5,400	70.9	2.9

III. Testing the model. Solutions of the equations were checked against results obtained empirically in order to determine the accuracy of the theoretical approach. In Table II are shown the concentrations of thiopental estimated to be present in jugular venous blood at various times after thiopental injection. The concentration meas-

*We wish to thank M. L. Rockoff, Computer Center, University of Pennsylvania, for aid in programming these equations for solution on a digital computer.

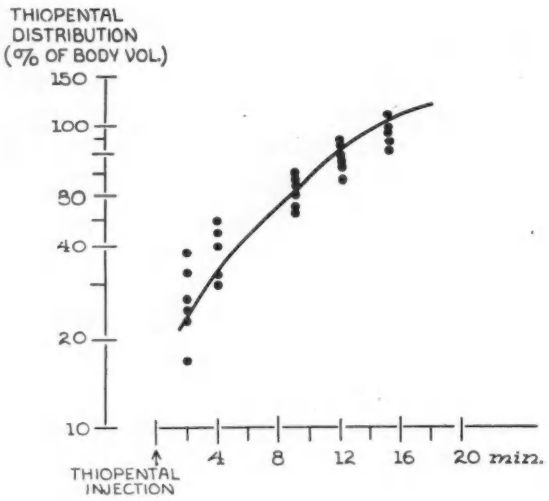


Fig. 2. Distribution of thiopental in the body at various times after its intravenous injection. (For explanation see text.)

ured (as described in the methods) at 1 minute was arbitrarily assigned a value of 100 per cent; concentrations measured at other times were expressed in terms of this value. Similar data were obtained from the mathematical solutions and these were compared with the measured values. The extent of the agreement between real and theoretical results is remarkable.

The distribution volume (as described in section I) was also calculated both from measured and mathematically predicted concentrations of thiopental in arterial blood. The measured data are those described in section I. Those used to predict concentrations mathematically are shown in Table I. Fig. 2 shows the extent of agreement between measured (dots) and calculated (line) data. The agreement is sufficiently close for the present purpose, although there is some difference among subjects, and a tendency for the volume calculated from measured concentrations initially to surpass and later to lag behind the theoretical one. Reasons for these discrepancies will be considered in another paper.

Table III shows the calculated and measured concentrations of thiopental in fat at various times after injection of the drug. The calculated values were derived from

Table II. *Concentrations of thiopental in internal jugular venous blood*

No. cases studied	*Time after injection (min.)*	*Per cent of level at 1 minute after intravenous injection*		
		Predicted	*Measured*	
			(Average)	*(Range)*
6	1	100	100	100–100
6	2	90	81	73– 88
5	4	65	59	53– 66
3	8	40	36	34– 40
1	16	15	12	—
0	32	10	—	—

Table III. *Calculated and measured thiopental concentrations in human fat at various times after intravenous injection*

Time (min.)	*Concentrations relative to blood*		*Source of fat*	*Number of samples*
	Calculated	*Measured*		
10–15	0.9	0.7	Subcutaneous	4
16–20	1.4	1.2	Subcutaneous	3
21–25	1.8	1.4	Subcutaneous	3
			Omental	1

solution of the simultaneous equations just described. The measured values were obtained by analyzing samples of subcutaneous and omental fat excised at various times after intravenous injection of a single dose (150 to 300 mg.) of thiopental in 4 subjects undergoing operation. The extent of the agreement is good, although the fat which was examined took up only 80 per cent of what was theoretically predicted.

The three sets of comparisons between measured and predicted values which have been reviewed show a high order of agreement, and suggest that the model described in section II is adequate to predict the distribution of thiopental in the human body.

IV. Distribution of thiopental in the body after a single intravenous injection. Fig. 3 shows the distribution of a single dose of thiopental in the body at various times after its intravenous injection. The viscera (heart,

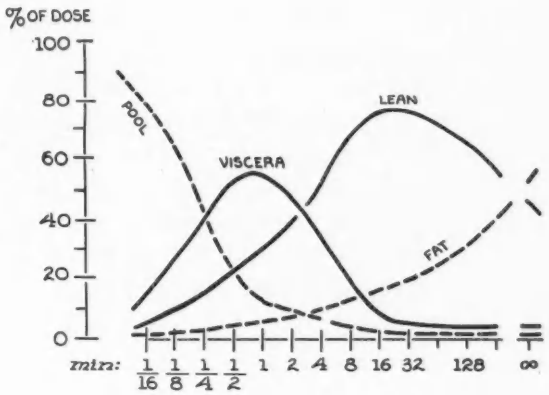


Fig. 3. Distribution of thiopental in different bodily tissues and organs at various times after its intravenous injection.

kidney, splanchnic area, and central nervous system) have been treated as a unit. All of the data in the figure were derived mathematically as described in section II. It should be noted that the time scale progresses geometrically, not linearly.

The figure shows that the thiopental in the central pool is soon depleted as the result, primarily, of uptake by the rapidly perfused visceral tissues. At 1 minute after injection, for example, the rapidly perfused viscera contain 55 per cent of the dose, although their combined mass is only 6 per cent of the body weight. The central pool now contains only 12 per cent of the original dose of thiopental, the fat 5 per cent, and the poorly perfused aqueous tissue 28 per cent. The thiopental which is at this moment so greatly concentrated in the rapidly perfused viscera is again redistributed as time passes, principally because it enters other aqueous tissue. Uptake by fat is relatively unimportant until much later. For example, at 30 minutes after the injection, the rapidly perfused tissues contain only 5 per cent of the injected dose, and the fat 18 per cent, while the poorly perfused aqueous tissues contain 75 per cent. In other words, the vital organs, including the brain, have by now given up 91 per cent of their peak content or half the total dose, but the fat has gained less than one fourth of this, while the poorly perfused aqueous tissues have gained more than three fourths. When equilibrium occurs (after more than 8 hours), fat will contain about 60 per cent of the injected dose, viscera 4 per cent, and the other aqueous tissues the remainder. Thus, by 30 minutes after the injection, the viscera will have proceeded 96 per cent of the way toward their final thiopental content as the result, predominantly, of drug uptake by the slowly perfused aqueous tissues. Fat acquires thiopental principally from the slowly perfused tissues and not from the viscera. For this reason, the percentage of body fat may not significantly affect the quantity of thiopental in the central nervous system under the conditions described. This is not to say that the total quantity of fat is unimportant, because an increase in the total perfused body mass from any cause, without increase in the visceral mass, would result in an increased early rate of withdrawal of thiopental from the central nervous system. The early effect would occur, however, if the added tissue were muscle, bone, or fat, because it depends more upon tissue blood flow and mass than upon the affinity of the tissue for thiopental. Fat exerts its full ability to concentrate thiopental much later (more than 8 hours after injection). The proportion of fat in the body would thus be of more consequence long after massive single doses or after long infusions, i.e., when all the watery tissues were saturated with the drug.

To test these predictions further, the percentage of an injected dose of thiopental calculated to be in the central nervous tissues of a normal person (70 Kg., 15 per cent fat), was compared with that estimated for an obese person (85 Kg., 30 per cent fat). At 1 minute after the injection, the central nervous system content of thiopental was approximately 14 per cent of the dose in both cases. At the end of 1 hour, the central nervous system was estimated to contain 1.27 per cent of the dose in the normal person, and 0.83 per cent in the obese person. The quantity of thiopental *removed* from the nervous system in 1 hour thus was 94 per cent of the peak content in the obese

person and 91 per cent in the normal person. About half of this difference is attributable to the greater mass of the obese person. The other half is attributable to the fact that the excess tissue was fat, rather than muscle, connective tissue, or bone.

These observations are not surprising because fat, although high in thiopental affinity, is poorly perfused. The rate of perfusion *per gram* is nearly the same in fat as in resting muscle,[5] but the rate of perfusion *per unit of dilution capacity* (mass times affinity) is low; therefore, fat cannot come into equilibrium with fat-soluble substances contained in arterial blood for a long period. Consequently, the concentration of thiopental in fat builds up slowly. Under the conditions considered, fat contained only half its equilibrium concentration 2 hours after injection of the drug.

Brodie and his associates[2] observed similar saturation rates in their work with dogs and commented upon the slowness with which various fat depots attained a uniform concentration of thiopental. Their interpretation differs from ours largely because they considered fat to be the only tissue capable of depleting the viscera of thiopental. Their data, however, like ours, indicate that the concentration of thiopental in muscle increased slowly to attain a maximum at about 20 minutes after thiopental injection. They did not interpret this finding. We interpret it as supporting our belief that the uptake of thiopental by lean body tissues is very important in depleting the visceral organs (e.g., brain) of thiopental.

Another consequence of the slow and persistent uptake of thiopental by fat is that it could cause an overestimate of the rate at which thiopental is metabolized. Brodie and his associates[3] considered that the rate of disappearance of thiopental from the blood at times greater than 1 or 2 hours after injection was equal to the metabolic rate. According to our data, one third to one half of the rate of disappearance noted by these authors, and attributed to metabolism, was actually caused by continuing deposition of the drug in fat. This finding reinforces their conclusion that the metabolism of thiopental occurs slowly. It must be even slower than their estimate would indicate. It minimizes the role which should be ascribed to fat, however, for the rapidity of fat uptake appears to be no greater than the rate of metabolic degradation. Accordingly, these two processes should be of equal (and small) importance in limiting the duration of narcosis after a single intravenous injection of thiopental.

References

1. Bazett, H. C., and Bard, P.: The Circulation, in Bard, P., editor: Part I, Medical Physiology, ed. 10, St. Louis, 1956, The C. V. Mosby Company.
2. Brodie, B. B., Bernstein, E., and Mark, L. C.: The Role of Body Fat in Limiting the Duration of Action of Thiopental, J. Pharmacol. & Exper. Therap. **105**:421, 1952.
3. Brodie, B. B., Mark, L. C., Papper, E. M., Lief, P. A., Bernstein, E., and Rovenstein, E. A.: The Fate of Thiopental in Man and a Method for Its Estimation in Biological Material, J. Pharmacol. & Exper. Therap. **98**:85, 1950.
4. Habif, D. V., Papper, E. M., Fitzpatrick, H. F., Lowrance, P., Smythe, C. McC., and Bradley, S. E.: The Renal and Hepatic Blood Flow, Glomerular Filtration Rate, and Urinary Output of Electrolytes During Cyclopropane, Ether, and Thiopental Anesthesia, Operation, and the Immediate Postoperative period, Surgery **30**:241, 1951.
5. Jones, H. B.: In Glasser, Otto, editor: Medical Physics, Chicago, 1950, The Year Book Publishers, Inc., vol. 2, p. 855 ff.
6. Kety, S. S., and Schmidt, C. F.: The Determination of Cerebral Blood flow in Man by the Use of Nitrous Oxide in Low Concentrations, Am. J. Physiol. **143**:53, 1945.
7. Keys, A., and Brozek, J.: Body Fat in Adult Man, Physiol. Rev. **33**:245, 1953.
8. Petrakis, N. L., Masouredis, S. P., and Miller, P.: Local Blood Flow in Human Bone Marrow in Leukemia and Neoplastic Diseases as Determined by the Clearance Rate of Radioactive I^{131}, J. Clin. Invest. **32**:952, 1953.
9. Price, H. L., Dundee, J. W., and Conner, E. H.: Rates of Uptake and Release of Thiopental by Human Brain; Relation to Kinetics of Thiopental Anesthesia, Anesthesiology **18**:171, 1957.
10. Wechsler, R. L., Dripps, R. D., and Kety, S. S.: Blood Flow and Oxygen Consumption of the Human Brain During Anesthesia Produced by Thiopental, Anesthesiology **12**:308, 1951.

Effects in man of single and combined oral doses of reserpine, iproniazid, and D-lysergic acid diethylamide

The use of d-lysergic acid diethylamide, LSD, as a reference standard for the evaluation of psychopathologic symptoms was tested. A group of healthy male volunteers took weekly doses of LSD in order to become familiar with the temporary mental changes induced by the drug.

The subjects were able to identify three dose levels of LSD and to differentiate LSD from placebo and other drugs. A standard curve of the responses to LSD could be constructed for these subjects.

A combination of reserpine plus iproniazid was found not to produce LSD-like psychopathologic symptoms. The addition of reserpine and iproniazid to LSD did not change the degree or intensity of LSD-induced psychopathologic symptoms in man.

Edmund W. J. DeMaar, M.D.,* Harry L. Williams, M.D., A. I. Miller, M.D., and C. C. Pfeiffer, M.D. *Emory University, Ga.*
Department of Pharmacology, Emory University

The psychic effects of *d*-lysergic acid diethylamide (LSD) mimic certain psychopathologic symptoms which are observed in psychiatric conditions such as schizophrenia. Reserpine treatment diminishes the psychotic symptoms of some schizophrenic individuals, but, when administered orally, does not antagonize the mental effects of LSD in normal man.[7] A possible difference may thus exist in the action of reserpine on these two types of psychopathologic reactions.

Reserpine decreases the content of norepinephrine and 5-hydroxytryptamine (5-HT) in the rabbit brain.[2,6] The normal destruction of 5-HT is inhibited by the monoamine oxidase inhibitor iproniazid.[10] The combined administration of reserpine and iproniazid has been reported to produce LSD-like symptoms in the rabbit.[9] These symptoms have been attributed to an increase in the free 5-HT in the brain, resulting from its release by reserpine and the subsequent prevention of its destruction by iproniazid. For these reasons, it has been suggested that free 5-HT may be

This study was supported in part by Grant M-875 from the National Institute of Mental Health, National Institutes of Health, United States Public Health Service, and a grant from the Geschickter Fund for Medical Research, Inc.

*Present address, Merck Sharp & Dohme Research Laboratories, West Point, Pa.

involved in the development of psychopathologic symptoms in man. The combined administration of 11-desmethoxyreserpine and iproniazid, however, decreases the severity of the psychopathologic symptoms of schizophrenic patients.[4] The effects of the drug combination, reserpine plus iproniazid, on normal man have not been reported.

We have compared, in normal man, the mental and physical effects of the drug combination, reserpine plus iproniazid, with those of LSD. To study the possibility of a decrease or an increase of the LSD-effect by reserpine plus iproniazid, the combination of all three drugs also has been employed. The use of LSD as an investigative tool in psychopharmacology requires a method of mathematical measurement of the mental response. The effect of LSD is experienced by a human subject in various ways. Subjects report changes in mood, either in the direction of euphoria or depression, anxiety and nervousness in the form of muscular stiffness and "inner tremors," difficulties in concentration and in orientation in time, feelings of strangeness and dreamlike states. Other changes are alterations in sensory modalities, distortions of the body image, changes in the appreciation of taste, smell, and sound, and a number of visual phenomena which include blurring of vision, perceptual changes in distance, size, shape, and color, and the appearance of kaleidoscopic patterns.

Conventional psychometric tests do not measure these alterations in status and performance of the mind. Questionnaires can be used on untrained subjects to distinguish between LSD and placebo,[1] but cannot be used to distinguish significantly between dose levels of LSD with 25 μg intervals. When a subject has received LSD several times, apparently he then becomes able to identify the drug and to quantify the dose given with a high degree of precision.[3,8] In this study we have relied on these subjective reports by trained subjects. The experimental design employed rigidly controlled and standardized conditions to make this subjective evaluation as uniform as possible.

Methods

Selection of subjects. The experimental subjects were a group of 16 volunteers, all inmates of the U.S. Federal Penitentiary in Atlanta, Georgia. They were in good physical health, their mean age was 34 years, and their mean weight was 160 pounds. A Rorschach test was performed on them to detect subjects with latent psychotic tendencies. The latter as well as emotionally unstable individuals were excluded from the study. Of the subjects chosen, the I.Q. ranged from 90 to 123, and the "psychopathic deviant" scores of the Minnesota Multiphasic Personality Inventory had a mean of 68.1, with a range from 50 to 90. Except for character disorders, no psychiatric abnormalities were present in these subjects.

Administration of drugs. Treatments were administered orally, 2 hours after a light breakfast, once a week. A double-blind control was used. Each time 50 ml. quinine-flavored liquid and four tablets were given. The treatments tested were: 2 mg. reserpine with 100 mg. iproniazid; LSD in doses of 25, 50, and 100 μg; LSD in doses of 25 or 50 μg with 2 mg. reserpine and 100 mg. iproniazid; 2 mg. reserpine; 100 mg. iproniazid; and LSD in doses of 25 or 50 μg in combination with either 2 mg. reserpine or 100 mg. iproniazid.

Procedures. The subjects were divided into 8 groups of 2 each. All groups were tested in a hospital room with 4 groups of subjects present each time. The subjects were encouraged to stay in bed during the trials. The objective signs of drug effect were measured and recorded by one of the physicians participating in the project. These included hourly measurements of blood pressure, pulse rate, pupil diameter, oral temperature, and hand steadiness. The latter was measured with a technique which required the subject to introduce a round metal stylus into a hole with a di-

ameter of 2, 3, or 4 mm. The subject was instructed to hold the stylus in the hole for a period of 30 seconds and to avoid contact with the edges. The number of contacts for each hole was expressed in counts (errors) per 30 seconds. An increase in the number of counts indicated a decrease in hand steadiness. The scores provided only a rough estimate of the steadiness.

The subjective mental effects were assayed on the basis of an interview with the subjects on their experiences 3 hours after medication. Each subject had experienced during his training the effects of single doses of 25, 50 (either one of them twice), and 100 μg LSD, at least 4 times. He was able to identify typical symptoms of the LSD effect and had learned to associate the number and the intensity of symptoms with particular doses of LSD. During the interview the subject was asked to identify his symptoms with the help of a series of questions.[1] The questions were arranged in an order of decreasing effects with those at the highest dose level appearing at the top and those with placebo at the bottom. Questioning was started alternately at the top or at the bottom of the list. The list follows:

1. Are things moving around you?
2. Do you have "funny" feelings on your skin?
3. Do you feel unsteady?
4. Do you feel as if in a dream?
5. Do you tremble inside?
6. Is there pressure in your ears?
7. Do you have difficulty in focusing vision?
8. Do you feel weak?
9. Do your hands and feet feel light or peculiar?
10. Is your skin sensitive?
11. Is salivation increased?
12. Is your appetite increased?
13. Do you feel ill in any way?
14. Do your hands and feet feel heavy?
15. Are you nauseated?
16. Do you have a "funny" taste in your mouth?
17. Do things seem too far away?
18. Is your hearing more acute than usual?
19. Do things seem too close?
20. Are your palms dry or cold?
21. Do you see double?
22. Does light bother you?
23. Are you cold?
24. Do you feel fatigued?
25. Is your appetite decreased?
26. Are you anxious?
27. Does your head ache?
28. Do you feel drowsy?

Finally the subject was asked whether he thought he had received LSD, and if so, what dose. The subject had a choice of six dose levels: 0, 25, 50, 75, 100, and over 100 μg. The dose level stated was recorded and used in the assays as the quantitative value of the drug response to LSD.

Twenty-four hours after the administration of the drugs, the subjects prepared a written statement in which they compared the duration of the mental effects of this drug with the effect of the drug given the previous week and indicated whether there were any prolonged effects beyond 3 hours after medication. After they had prepared their written statement, the subjects were told the drug and the dose administered. This learning process increases their sophistication for discerning the mental effects of the drug.

Design. The main data in these experiments were collected from 2 blocks of treatments. They were designed with 4 objectives in mind:

1. Do the subjects discriminate between LSD and other drugs?
2. Do the subjects discriminate between doses of LSD?
3. Does the combination of iproniazid plus reserpine induce LSD-like effects?
4. Does the addition of reserpine and iproniazid to LSD alter the number and the intensity of the psychopathologic symptoms?

Before the first block of treatments was started, the subjects were acquainted with the mental and physical effects of a 100 mg. dose of iproniazid and 2 and 4 mg.

Table I. *Subjects' groups*

Trial	*1 and 5*	*2 and 6*	*3 and 7*	*4 and 8*
1	Reserpine 2 mg. + Iproniazid 100 mg.	LSD 25 μg	Reserpine 2 mg. + Iproniazid 100 mg.	LSD 50 μg + Iproniazid 100 mg.
2	LSD 50 μg	Reserpine 2 mg. + Iproniazid 100 mg.	LSD 50 μg + Iproniazid 100 mg.	Reserpine 2 mg. + Iproniazid 100 mg.
3	LSD 25 μg + Iproniazid 100 mg.	Reserpine 2 mg. + Iproniazid 100 mg.	LSD 25 μg	LSD 50 μg
4	Reserpine 2 mg. + Iproniazid 100 mg.	LSD 25 μg + Iproniazid 100 mg.	Reserpine 2 mg. + Iproniazid 100 mg.	Reserpine 2 mg. + Iproniazid 100 mg.

Table II. *Subjective identification of LSD*

Medication received	*Reported as*	
	LSD	*No LSD*
LSD	15	0
Reserpine + iproniazid	3	24
LSD + iproniazid	14	3
Iproniazid	1	8

doses of reserpine. Following iproniazid alone, 4 subjects reported no effects at all, and 5 reported flush and warmth associated with a feeling of well-being or relaxation. One subject identified iproniazid once as a dose of 50 μg LSD. The reported side effects of reserpine were diffuse headache, nasal stuffiness, and facial flush. Doses of 25, 50, and 100 μg LSD were included in the treatments to maintain the sophistication of the subjects for the effects of LSD.

Results

In the first block of 4 treatments (Table I), the combination reserpine-plus-iproniazid was matched with LSD in doses of 25 or 50 μg, and similar doses of LSD-plus-iproniazid. The objective was to test whether the combination of iproniazid-plus-reserpine induced an LSD-like effect.

The combination of 2 mg. reserpine-plus-100 mg. iproniazid was given 27 times. The LSD-like effect was reported only 3 times. All subjects indicated feelings of warmth, showed facial flushing and conjunctival injection. Later they reported congestion of the nasal mucosa. Feelings of drowsiness or relaxation were reported 5 times. Five trials were missed.

Inspection of the scores made for identification of LSD and the scores made for the combination of reserpine and iproniazid shows that the subjects are able to discriminate between LSD and other drugs and, therefore, that there is no reason to assume that an LSD-like effect is induced by the reserpine-iproniazid combination in these subjects (Table II).

The physical findings do not reveal any similarities in man between LSD and this

combination in the doses and route used. The combination does not induce the most constant LSD effect, dilatation of the pupil. There is a lowering of systolic and diastolic blood pressure, while LSD induces a rise in systolic blood pressure.

This first testing procedure gave information about the absence or presence of an LSD effect in the range from zero to a 25 or 50 μg dose. A smaller LSD-like effect may be detectable by testing for an additive, potentiating, or antidotal effect of reserpine and iproniazid on the LSD responses. This method may be more sensitive. A second block of treatments was set up with these objectives in mind (Table III).

The objectives 2 and 4 were now considered. Do the subjects discriminate between doses of LSD? Furthermore, does the addition of reserpine and iproniazid to LSD change the number and the intensity of the psychopathologic symptoms? For the evaluation of the presumed change a standard dose response curve of the group, acting as a whole, for the mental effects of LSD had to be determined.

In a quantitative method of evaluation the estimated doses for LSD alone at the three dose levels have been pooled and their means plotted against the log of the administered doses. These 3 points can be joined by a straight line (Fig. 1). Table IV gives the means of the values obtained. Table V gives the results of the analysis of variance on the data, including the controls, obtained from the administration of LSD alone. The log-dose response rela-

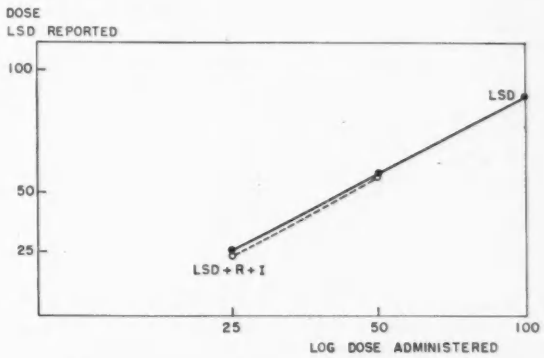


Fig. 1. Response curves of the quantitative estimation are plotted for *d*-lysergic acid diethylamide alone and in combination with reserpine, 2 mg., plus iproniazid, 100 mg., on a semilogarithmic scale. The ordinate is the dose of LSD recorded. The abscissa is the actual dose administered plotted on a logarithmic scale.

Table III. *Subjects' groups*

Trial	*1 and 3*	*2 and 4*	*5 and 8*	*6 and 7*
1	LSD 25 μg	LSD 50 μg	LSD 100 μg	Reserpine 2 mg. + Iproniazid 100 mg.
2	Reserpine 2 mg. + Iproniazid 100 mg.	LSD 25 μg + Reserpine 2 mg. + Iproniazid 100 mg.	LSD 50 μg + Reserpine 2 mg. + Iproniazid 100 mg.	LSD 50 μg
3	LSD 25 μg + Reserpine 2 mg. + Iproniazid 100 mg.	LSD 25 μg	LSD 50 μg	LSD 50 μg + Reserpine 2 mg. + Iproniazid 100 mg.
4	LSD 50 μg	Reserpine 2 mg. + Iproniazid 100 mg.	LSD 25 μg + Reserpine 2 mg. + Iproniazid 100 mg.	LSD 100 μg

Table IV. *Quantitative evaluation of LSD*

Medication received	*Mean of doses reported and standard error of the mean*
LSD 25 μg	26.3 ± 3.8
LSD 50 μg	57.9 ± 3.9
LSD 100 μg	91.1 ± 8.5
LSD 25 μg + Reserpine 2 mg. + Iproniazid 100 mg.	24.3 ± 6.3
LSD 50 μg + Reserpine 2 mg. + Iproniazid 100 mg.	57.3 ± 3.2

Table V. *Analysis of variance of the results of experiments to show the ability of the group to identify doses of d-lysergic acid diethylamide*

Source of variation	*SS*	*df*	*MS*	*F*	*P*
Curvature	46445.20	2	23222.60	55.2	0.05
Linear regression	71427.80	1	71427.80	169.5	0.05
Doses	117873.00	3	39291.00	93.3	—
Error	50944.46	121	421.03	—	—
Total	168817.46	124	—	—	—

tionship is significantly linear, $P>0.01$ and <0.05. The curve is straight from 25 to 100 μg. There is a curvature from zero to 25 μg. All three errors were pooled because the variations due to subjects and the variations due to the interaction between doses and subjects were not larger than the variation between replicate tests using the same dose on the same subject.

A relationship does exist between the doses administered and the doses reported for the group acting as a whole. Therefore, the standard curve in Fig. 1 can be used as a measure of the psychic effects of LSD in graded doses. It shows that this group of subjects is able to identify the intensity of psychopathologic symptoms and to correlate these symptoms with a certain dose of LSD.

The number and the intensity of the psychopathologic symptoms induced by 25 and 50 μg of LSD as shown in this assay and those induced by the combination of LSD plus reserpine and iproniazid were compared. There was no significant difference between these two curves and hence between these two treatments (Fig. 1). No difference in duration of the effects was revealed by the written statement.

The effects of LSD either in combination with reserpine only or with iproniazid only also were tested in 10 and 17 trials, respectively, to exclude the possibility that the resultant LSD-combination curve was the algebraic sum of two gross opposite effects. The mean reported doses of these two treatments did not differ from the LSD-standard values.

In a final evaluation of the results of all trials the objective was to determine whether subjects can discriminate between LSD and other drugs. The results of 172 trials were available for analysis. Of this total, 122 were administrations of LSD, either alone in doses of 25, 50, or 100 μg or in combination with other drugs. The remaining 50 were administrations of other drugs alone. In 122 administrations of LSD, the subjects reported an LSD-like effect 113 times. "No LSD" was reported 49 times. Analysis by chi-square test of the scores gave a value of 116.8, $P<0.001$. Thus, it was established that the ability of the subjects to discriminate between the treatments was highly significant.

Discussion

The use of *d*-lysergic acid diethylamide as a reference standard for the evaluation of psychopathologic symptoms was examined. Within the range of dose levels of 0, 25, 50, and 100 μg, a group of subjects was able to establish a relationship between the doses administered and the doses of LSD reported. The measurement of such a highly individual response as the amount and degree of intensity of psychopathologic symptoms based on the estimation of the dose administered has the advantage of being simple and rapid. The highly significant value for the difference obtained in identification scores between LSD and other drugs indicates usefulness of LSD in an assay of this type of psychic activity.

An estimate of how many of these responses in general are required for a reliable assay, however, cannot be given for these data. In this study the error of the assay has been small, partly because of the inclusion of a large number of zero responses on zero dose. The number of responses required will of course depend on such variables as the background and training of the subjects. The maintenance of a constant group of well-trained subjects makes this technique difficult, unless special experimental conditions such as a closed ward or penitentiary are available. Even in spite of the rigidly controlled experimental conditions some subjects were absent at one or two trials and two had to be replaced during the course of these experiments. These exclusions disrupted the Latin square design of the experiment and therefore no information on possible changes in drug or subject responses with time was obtained.

In previous studies atropine, niacin, and 2-brom-*d*-lysergic acid diethylamide were shown to be ineffective as modifiers of the LSD response in man.[8] Chlorpromazine[7] and amobarbital were reported to diminish the response.[5] In this study the combination of reserpine and iproniazid did not alter the development of LSD-like psychopathologic symptoms.

In the quantitative assay of this study identical curves were obtained for LSD alone and in combination with doses of reserpine and iproniazid. Therefore, these treatments were similar in their psychic effects. The conclusion may be drawn that there was no extensive change by the action of reserpine plus iproniazid on LSD-induced psychopathologic symptoms, which would be expected if free 5-HT were related to or interfered with the development of LSD psychopathologic symptoms. The results of the present study fail to support this concept.

Conclusions

1. The subjective and physical effects of the drug combination reserpine plus iproniazid have been compared with the effects of *d*-lysergic acid diethylamide on trained healthy male volunteers.
2. Evaluation of psychic effects has been based on interviews 3 hours after drug administration and on written statements prepared 24 hours later.
3. Subjects can discriminate between the mental effects of LSD and those of the drug combination and are able to estimate the three dose levels of LSD administered.
4. The scores for qualitative identification of LSD and the combination of reserpine plus iproniazid indicate that the psy-

chic and physical effects of these two treatments are dissimilar. Therefore, the effects of the combined administration of reserpine plus iproniazid do not mimic those of LSD.

5. The log-dose response curves for the dose identification of the two dose levels of LSD and the two dose levels of LSD plus reserpine and iproniazid show that these treatments do not differ in their psychic effects. The combination of reserpine plus iproniazid neither increases nor decreases the degree and intensity of LSD-induced psychopathologic symptoms in man.

References

1. Abramson, H. A., Jarvik, M. E., Kaufman, M. R., Kornetsky, C., Levine, A., and Wagner, M.: Lysergic Acid Diethylamide (LSD-25). 1. Physiological and Perceptual Responses, J. Psychol. **39**:3-60, 1955.
2. Brodie, B. B., Shore, P. A., and Pletscher, A.: Serotonin-Releasing Activity Limited to Rauwolfia Alkaloids With Tranquillizing Action, Science **123**:922-993, 1956.
3. DeMaar, E. W. J., Miller, A. I., and Williams, H. L.: Psychic Effects of Combined Administration of Rauwolfia Alkaloids and Iproniazid, Fed. Proc. **16**:291, 1957.
4. Gallagher, W. J., Berry, J. M., Durden, W. D., and Lazenby, W. D.: Comparative Study of Reserpine, 11-Desmethoxyreserpine, and Rauwolfia Alkaloids in Treatment of Schizophrenia, in Tranquilizing Drugs, Washington, D.C., 1957, American Association for the Advancement of Science, pp. 133-147.
5. Hoch, P. H.: Production and Alleviation of Mental Abnormalities by Drugs. Abstracts of Reviews, Twentieth Int. Phys. Congress, Brussels, 1956, pp. 429-442.
6. Holzbauer, M., and Vogt, M.: Depression by Reserpine of the Noradrenaline Concentration in the Hypothalamus of the Cat, J. Neurochem. **1**:8-11, 1956.
7. Isbell, H.: Effect of Chlorpromazine, Reserpine and Frenquel on LSD Reaction, Fed. Proc. **15**:442-443, 1956.
8. Miller, A. I., Williams, H. L., and Murphree, H. B.: Niacin, Niacinamide or Atropine Versus LSD-25 Model Psychoses in Human Volunteers, J. Pharmacol. & Exper. Therap. **119**:169-170, 1957.
9. Shore, P. A., and Brodie, B. B.: LSD-like Effects Elicited by Reserpine in Rabbits Pretreated With Iproniazid, Proc. Soc. Exper. Biol. & Med. **94**:433-435, 1957.
10. Zeller, E. A., Barsky, J., Fouts, J. R., Kirchheimer, W. F., and Van Orden, L. S.: Influence of Isonicotinic Acid Hydrazide (INH) and 1-Isonicotinyl-2-Isopropyl Hydrazide (IIH) on Bacterial and Mammalian Enzymes, Experientia **8**:349, 1952.

The clinical use of an epoxide alkylating agent, epoxypropidine, in neoplastic disease

In a study of a series of synthetic bis-epoxide alkylating agents, a correlation was found between base strength and antileukemic effectiveness. Epoxypropidine (1,1'-bis (2,3 epoxypropyl)-4,4'-dipiperidyl) was the most active analog in this regard and, in addition, possessed physical characteristics desirable for a pharmacologic agent. Preclinical trials revealed greater antitumor activity on a weight-for-weight basis than that of an analog of the same series which had been demonstrated to be of clinical usefulness. Epoxypropidine was used clinically in the treatment of 21 patients with neoplastic diseases. Objective response and significant clinical improvement were noted in patients with Hodgkin's disease, lymphosarcoma, reticulum cell sarcoma, and chronic myelogenous leukemia. Clinical experience with this agent in a dose causing comparable leukopenia was similar to that with another alkylating agent of the epoxide class, diepoxypropylpiperazine, and with nitrogen mustard. Epoxypropidine caused none of the visual aberrations seen with diepoxypropylpiperazine and, in divided doses, less nausea and vomiting than are seen with comparable doses of nitrogen mustard. Despite the greater activity by weight of this compound compared to the piperazine analog, the therapeutic index was not improved. Although compounds of greater clinical convenience have been devised, the general experience with alkylating agents to date argues against the probability of increasing anticancer activity by altering prosthetic groups.

Daniel G. Miller, M.D., Henry D. Diamond, M.D., F.A.C.P., and Lloyd F. Craver, M.D., F.A.C.P. *New York, N.Y.*

In the search for alkylating agents with greater tumor specificity or a higher therapeutic index, analogs of the ethylene imine and sulfonoxy methane classes have been extensively investigated.[5] The biologic significance of epoxides as alkylating agents was first described by Ross[8] in 1950, and since that time a limited number of reports have appeared on the therapeutic usefulness of members of this class of compounds.[6,7,10] In a previous paper,[7] the results of therapy with diepoxypropylpiperazine have been presented. This report deals with the dosage, method of administration, results, and side effects observed in the treatment of patients with neoplastic disease with diepoxypropylbipiperidyl (1,1'-bis (2,3 epoxypropyl)-4,4'-dipiperidyl), which hereafter will be referred to by the generic name epoxypropidine.

From the Lymphoma Service, Department of Medicine, Memorial Center for Cancer and Allied Diseases; the Lymphoma Section of the Division of Clinical Chemotherapy, Sloan-Kettering Institute for Cancer Research; and the Department of Medicine, Cornell University Medical College, New York, N.Y.

This work was covered by a grant from the National Cancer Institute (CY-3215).

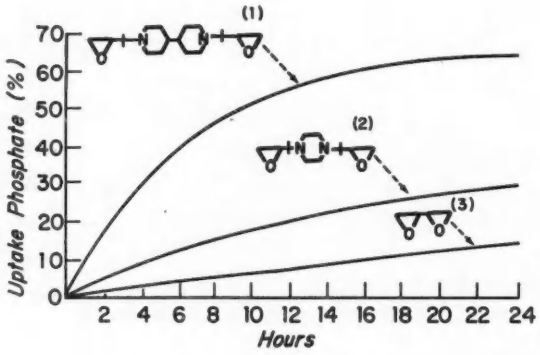


Fig. 1. Reaction of bis-epoxides with KH_2PO_4-K_2HPO_4 mixtures at pH 7 - 8; (1) diepoxypropylbipiperidyl (epoxypropidine), (2) diepoxypropylpiperazine, (3) diepoxybutane. (Adapted from Gerzon and associates.[2])

Gerzon and colleagues[2] in a study of a group of diamine bis-epoxides observed a correlation between the base strength of the bis-epoxides and their antileukemic effectiveness. This is a confirmation of the statement by Ross[8] that ". . . this type of compound would be expected to react more readily with nucleophilic centers in regions where acidity is relatively high. This might be significant from the standpoint of selective action towards different tissues." Other factors found to influence the biologic activity of this group of compounds were steric configuration, intrinsic molecular stability, and solubility in water.

Gerzon[2] suggested that a comparison of the rates of alkylation of the epoxy compounds with anions at a pH in the physiologic range might correlate with biologic activity. He chose a study of phosphorolysis because of the biologic significance attributed to the interaction of alkylating agents with the phosphate groups of nucleotides.[9] Fig. 1 is a diagram of the comparative rates of reaction of three epoxide compounds, with epoxypropidine showing greater reactivity than diepoxypropylpiperazine or diepoxybutane. Furthermore, there was a distinct parallel between the chemical reactivity toward the phosphate ion and the antileukemic effect of these compounds on mouse leukemia P 1534 as determined by Johnson and Wright.[3] Burchenal and coworkers[1] studied the effectiveness of epoxypropidine against a spectrum of mouse leukemias and, in general, found it as effective as diepoxypropylpiperazine in approximately one-tenth the dose.

Materials and methods

Epoxypropidine was supplied as a white crystalline powder in 50 mg. vials. This was readily dissolved in normal saline in a concentration of 10 mg. per milliliter and injected rapidly into the tubing of a fast-running infusion of 5 per cent glucose in distilled water.

A dose of 5 mg. per kilogram of body weight was observed to induce leukopenia and thrombocytopenia of the degree and duration ordinarily seen with 0.4 mg. per kilogram of nitrogen mustard. Smaller doses, 2 to 3 mg. per kilogram, were used in patients whose marrow function was compromised for any reason. When used in divided doses, 1 mg. per kilogram daily, epoxypropidine was not given for more than 3 successive days, since it was noted in several patients that this dose schedule caused a hematologic response comparable to that seen with 5 mg. per kilogram given as a single dose.

A total of 30 patients received this drug. Twenty-one received adequate dosage and were observed long enough for evaluation; the results in these patients will be presented. By adequate dosage is meant a dose sufficient to cause a fall in leukocytes to less than 4,000 cells per cubic millimeter. Of the patients treated, all had neoplastic diseases not amenable to surgery, e.g., Hodgkin's disease, lymphosarcoma, reticulum cell sarcoma, mycosis fungoides, chronic myelogenous leukemia, disseminated carcinomatosis.

A response was recorded after treatment with epoxypropidine when a measurable area of disease decreased by one-third or more provided no other area of disease was increasing at this time. Temperature response, x-ray, hematologic and biochemical studies were also used in appropriate situations as objective evidence of response. The

response of the disease was correlated with the response of the patient by an estimation of his performance status.[4]

Results

Table I presents the number of patients in each disease category and the results of therapy.

Hodgkin's disease. Nine patients with Hodgkin's disease were treated with epoxypropidine. In 6 there was objective evidence of response; 7 patients had diminution of symptoms with improvement of their performance status.

The types of response were as follows: A lowering of temperature was seen in 4 of the 5 patients with fever. The duration of improvement was 4, 7, and 8 weeks. One patient (U. S.) continues improved after 12 weeks. Other objective evidence of responses in this group of patients included decrease in lymphadenopathy, hepatomegaly, splenomegaly, and elevation of hemoglobin. No significant effect on pleural effusion was noted in 2 patients or on pulmonary infiltration in a third. Skin infiltrations in one patient with Hodgkin's disease failed to show any response to therapy.

Subjective responses included amelioration of chills, fever, night sweats, pruritus, bone pain, abdominal pain, and weakness. A diminution of such symptoms was usually accompanied by improvement in the performance status. Such improvement was seen in 7 of the 9 patients treated; the duration of improvement varied from 1 to 5 months.

Two patients with pruritus were treated to leukopenic levels. Patient C. C. had had a relapse after treatment with chlorambucil; many infected scratch papules were evident. Pruritus ceased within a week of treatment and the infected skin lesions healed. The itching returned after a symptom-free period of approximately 20 weeks. Patient F. G. had a 2 month symptom-free period following 0.4 mg. per kilogram of nitrogen mustard. When pruritus returned he received radiation therapy directed through 4 abdominal ports with no relief. Treatment to leukopenic levels with epoxypropidine also failed to cause any improvement. After hematologic recovery the patient was retreated with 0.4 mg. per kilogram of nitrogen mustard without any improvement.

Chills and night sweats were abated in 5 patients presenting these symptoms. The duration of improvement was 4 to 8 weeks in 3 patients and continues after 6 to 12 weeks in 2 patients. The last 2 cases will be presented in detail.

U. S., a 30-year-old white woman with Hodgkin's disease of 4 years' duration (Fig. 2), had previously been treated with radiation to the mediastinal and peripheral nodes, and diepoxypropylpiperazine. She was treated for night sweats, fever, and pruritus with diepoxypropylpiperazine 1 month after completion of a pregnancy. There was subjective and objective im-

Table I. *Results of treatment with epoxypropidine*

Disease	*No. of patients treated*	*Objective response*	*Clinical improvement*
Hodgkin's disease	9	6	7
Lymphosarcoma	4	3	1
Reticulum cell sarcoma	3	3	3
Mycosis fungoides	1	0	0
Chronic myelogenous leukemia	1	1	1
Lung carcinoma	1	1	0
Ovarian carcinoma	1	0	0
Breast carcinoma	1	0	0
Total	21	14	12

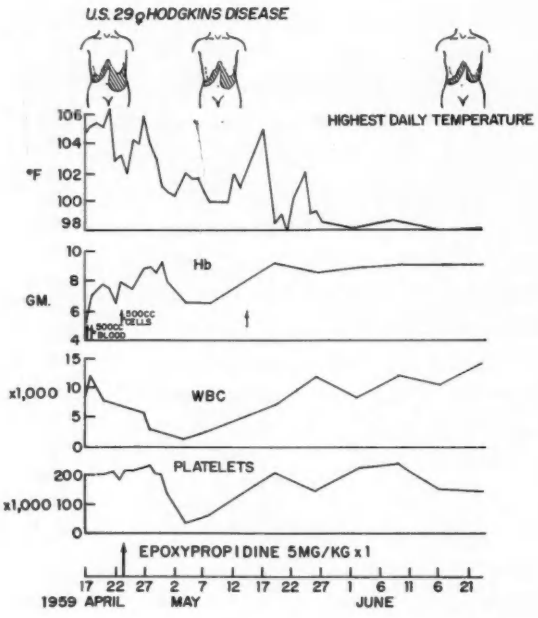


Fig. 2. Response of 29-year-old white woman with Hodgkin's disease to treatment with epoxypropidine.

provement for 13 months. Following a second pregnancy, the patient developed night sweats, chills, fever of 103° to 108° F., anemia, progressive hepatosplenomegaly, and lymphadenopathy. She was treated with 5 mg. per kilogram of epoxypropidine. There was marked symptomatic improvement with cessation of night sweats and increased ability to perform her daily tasks. There was a decrease in the size of the spleen from 20 to 11 cm. in the longitudinal diameter and decrease in lymphadenopathy. The need for transfusions decreased and the hemoglobin rose from the 4 to 6 Gm. level before therapy to the 9 to 10 Gm. range. The fever disappeared gradually and there were occasional febrile episodes for about 1 month. She was able to control the febrile recurrences with aminopyrine. However, as her improvement became established, she required less and less of the antipyretic agent and 1 month after treatment she required none. Studies of the red cell survival with radioactive chromium tagged red cells (performed by Dr. John Richmond) showed decreased survival time and splenic trapping. This was not improved after treatment although the patient's hemoglobin level rose. Eight weeks after receiving epoxypropidine, she was treated with radiation therapy through an upper abdominal port in an attempt to improve red cell survival and decrease splenic trapping. Red cell survival and distribution studies were repeated and these are still underway. It is now 12 weeks after epoxypropidine therapy and improvement of the previously described symptoms continues. In the last month the patient has developed signs of a polyneuritis from which she is recovering spontaneously.

C. B., a 32-year-old white woman with Hodgkin's disease of 6 years' duration, had previously been given radiation therapy through cervical, mediastinal, and abdominal ports, TEM, ACTH, and prednisone. At the time of treatment with epoxypropidine, the patient had been receiving prednisone, 20 mg. daily, for 1 month with no effect on her symptoms of fever, night sweats, anorexia, and weakness. On physical examination, the liver was 4 cm. below the costal margin and there was marked tenderness in the left lower quadrant (with negative barium enema and proctoscopy, and negative pelvic examination). The prednisone was continued in a dose of 15 mg. daily. She was given epoxypropidine, 1 mg. per kilogram daily for 3 days. Her symptoms abated rapidly, abdominal tenderness decreased, and only the liver edge was palpable. No hematologic depression occurred. One month later the patient developed bone pain in the lumbar spine. The x-ray examination was negative. She was given a second course of epoxypropidine, 3 mg. per kilogram in one dose. There was mild nausea for 24 hours and one episode of emesis. A chemical thrombophlebitis developed at the site of the drug injection. Within a week of treatment, symptoms decreased and the liver was no longer palpable. Three weeks later the patient noticed the onset of low-grade afternoon fevers and mild sweating. These persisted for 3 weeks and it was decided to give her a third course of epoxypropidine. A course of 3 mg. per kilogram was given with the same side effects as the

second course. The symptoms gradually decreased and for the past 6 weeks she has been free of fever or sweating. With the second and third courses of epoxypropidine, there has been no significant depression of hemoglobin, white blood cells, or platelets.

Lymphosarcoma. Four patients with lymphosarcoma received 5 courses of epoxypropidine. Three of the 4 showed objective evidence of improvement. One of these 3 patients also had significant clinical improvement, and a second patient was treated for progressive disease although he was symptom free. These 4 cases may be summarized as follows.

M. S. was a 60-year-old white woman with lymphosarcoma of 6 years' duration, the clinical extent of whose disease was hepatosplenomegaly, bone lesions, and enlarged mediastinal nodes. A continual hacking cough was the patient's chief complaint. Previous treatment included radiation therapy, nitrogen mustard, and chlorambucil. Recently prednisone, 15 mg. daily, had been given without significant improvement. The patient was treated with epoxypropidine, 1 mg. per kilogram daily for 3 days, with no unusual side effects. All palpable disease decreased and there was decrease of the mediastinal mass as well, with concomitant relief of the nonproductive cough. The cough returned after 14 weeks of improvement.

M. T. was a 68-year-old white woman with lymphosarcoma of 6 months' duration. Clinically the disease affected the liver, spleen, lymph nodes, and bone, and there was pleural effusion. Previous therapy included nitrogen mustard, and radiation with transient effect. The patient was treated with 3 mg. per kilogram of epoxypropidine with no untoward side effects. There was decrease in lymphadenopathy and hepatosplenomegaly but the patient's condition continued to deteriorate. Her course was complicated by a recent myocardial infarction, diabetes mellitus, and a draining abscess at the site of hip-pinning for a pathologic fracture.

B. Gi. was a 56-year-old white man with lymphosarcoma of 5 years' duration, whose disease clinically involved the liver and lymph nodes. The patient had previously had node, spleen, and liver response of 1 year's duration after a course of diepoxypropylpiperazine. At the time of treatment, the disease was progressing rapidly although the patient had no symptoms and there was no impairment of his performance status. He was treated with epoxypropidine, 5 mg. per kilogram. There was transient nausea for 24 hours, no emesis. No leukopenia or thrombocytopenia was induced. A decrease in lymphadenopathy took place. The liver which had been 3 cm. below the costal margin became impalpable. Improvement continues 10 weeks after therapy.

F. P., a 75-year-old white woman, had had lymphosarcoma for 4 months, clinically involving the peripheral and mediastinal lymph nodes with enlargement of the liver. There had been no previous treatment. Epoxypropidine was given in a dose of 1 mg. per kilogram daily for 3 days. There was no clinical effect and no leukopenia. Fifteen days later she was given epoxypropidine, 3 mg. per kilogram in one dose. Leukopenia occurred but only a slight softening of nodes was noted at the time of death (from a cerebral vascular accident) 13 days after the second course of treatment.

Reticulum cell sarcoma. Three patients with reticulum cell sarcoma were treated with epoxypropidine. All had striking subjective and objective improvement, but as is characteristic of this disease the duration of improvement was short lived.

M. R. was a 70-year-old white woman with reticulum cell sarcoma of 4 months' duration, clinically involving the liver and spleen with a painful disabling mass in the buttock. Therapy with epoxypropidine was begun 11 days after the patient completed a 6 weeks' course of radiation therapy, 3,000 r (in air) through 2 ports encompassing the mass. At the time of institution of epoxypropidine no improvement had been noted. Following treatment with this agent, 1 mg.

per kilogram daily for 2 days, objective and subjective improvement lasted for 7 weeks. The probability of a summative effect of radiation therapy and epoxypropidine on the mass in the buttock was most likely here.

J. K., a 61-year-old white man had reticulum cell sarcoma of 1 month's duration, extending clinically to the liver, spleen, peripheral nodes, and bone. There had been no previous therapy. He was treated with epoxypropidine, 1 mg. per kilogram daily for 2 days; 26 days later, 1 mg. per kilogram daily for 3 days. There were no untoward side effects; moderate leukopenia was induced (3,500 cells per cubic millimeter). The liver and spleen which were 10 and 8 cm., respectively, below the costal margin before treatment, decreased to 4 and 3 cm. below. There was also a decrease in lymphadenopathy. Weakness, itching, sweating, and fever which had distressed the patient were relieved. Seven weeks later there was relapse in the nodes, and 1 month after this the patient died of pneumonia in another hospital.

B. Go. was a 64-year-old white woman with reticulum cell sarcoma of 6 months' duration (Fig. 3). Clinically the disease manifested itself by enlarged peripheral nodes, subcutaneous masses, nodular pulmonary infiltrates, a diffuse intra-abdominal mass, and anemia. In addition, the patient had symptoms of a low-grade intestinal obstruction. Previously she had been treated by radiation to the local tumor masses. Epoxypropidine was given in a dose of 1 mg. per kilogram daily for 3 days without untoward side effects. The abdominal symptoms subsided. The need for transfusions diminished. The hemoglobin which had been in the 5 to 6 Gm. range rose to 9 to 10 Gm. levels. A 7 by 4 cm. right infrascapular mass became impalpable as did the liver and spleen which had been enlarged to 4 and 5 cm., respectively, below the costal margin prior to treatment. A response of the other parameters of disease was also recorded although less complete. Six weeks after treatment, the signs of intestinal obstruction recurred with rapid progression of pulmonary infiltration; 0.4 mg. per kilogram of nitrogen mustard failed to influence the course of the disease and the patient died 1 month later.

Miscellaneous. The results obtained in this group of miscellaneous malignant tumors may be summarized as follows.

R. T., a 40-year-old white woman with advanced mycosis fungoides, had a transient decrease of eczematoid lesions but no improvement of infiltrative, tumor, or ulcerative lesions which were the source of her distress.

A. S., a 41-year-old white woman with chronic myelogenous leukemia, received 6 injections of epoxypropidine, 0.5 mg. per kilogram, over a 4 week period. There was a decrease in the size of the spleen from 10 to 5 cm. and of the liver from 4 to 2 cm. below the costal margin. The need for transfusions ceased. The white count diminished from 339,000 to 15,806 cells per cubic millimeter. Another intravenous injection of 1 mg. per kilogram did not keep the white

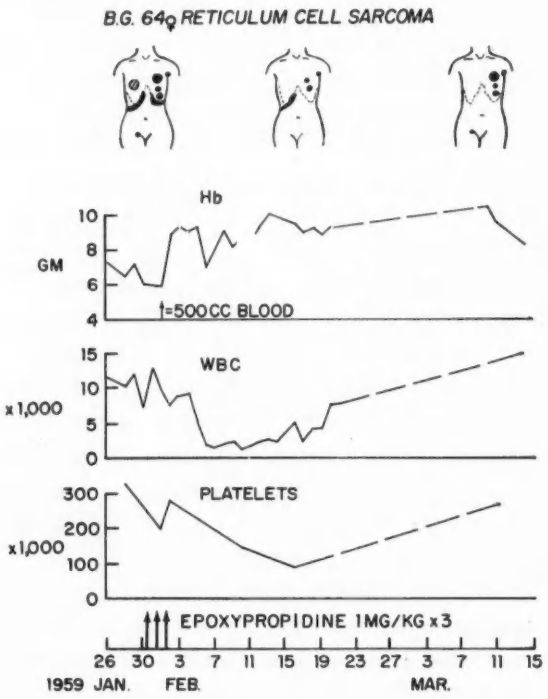


Fig. 3. Response of 64-year-old white woman with reticulum cell sarcoma to treatment with epoxypropidine.

Table II. *Side effects of treatment with epoxypropidine*

Daily dose	*No. of days given*	*Courses*	*Nausea and emesis*	*Thrombophlebitis*	*Leukopenia*
1 mg./Kg.	1, 2 or 3	13	0	0	7
2-3 mg./Kg.	1	10	4	5	9
5 mg./Kg.	1	5	2	0	4

blood cell count from increasing rapidly and the patient was treated with busulfan.

Three patients with disseminated carcinoma (lung, ovary, and breast) received 5 mg. per kilogram of epoxypropidine. Two of these patients with lung and ovarian carcinoma had a decrease in measurable local disease; however, the patient with ovarian carcinoma had advancing disease elsewhere. None of these 3 patients was clinically improved from this therapy.

Discussion

The results of therapy with epoxypropidine in these 21 patients are consonant with our experience with another epoxide alkylating agent, diepoxypropylpiperazine, and with nitrogen mustard. Although this compound is more active by weight than diepoxypropylpiperazine, in a dose that produces comparable leukopenia and thrombocytopenia there was no evident therapeutic advantage in this group of patients. Of interest are the 2 patients treated with epoxypropidine who had responded to diepoxypropylpiperazine approximately 1 year previously. Both had a satisfactory response to epoxypropidine (patients U.S. and B. Gi.).

The side effects of treatment with epoxypropidine are outlined in Table II. As with diepoxypropylpiperazine, nausea and vomiting appeared to be related to dosage. When the quantity of drug injected at one time did not exceed 1 mg. per kilogram, there were no nausea and vomiting and no thrombophlebitis. When the single injection was increased to 2 to 3 mg. per kilogram, 4 of 10 patients experienced nausea and vomiting and 5, thrombophlebitis. Increasing the dose to 5 mg. per kilogram did not increase the incidence of these side effects. The nausea and vomiting noted in all cases was mild, usually subsided in 24 hours, and seldom required treatment with sedatives or antiemetics. During the injection of epoxypropidine, most patients experienced a warm or flushing sensation and a taste of "gasoline or ether or alcohol." These reactions were in no case distressing. There were no instances of visual aberrations as occurred with diepoxypropylpiperazine. One patient had a grand mal seizure after injection of 3 mg. per kilogram of epoxypropidine but this patient had had such seizures after parenteral injections of a number of substances including sterile water.

Leukopenia, usually but not always accompanied by thrombocytopenia, occurred in 20 of the 28 courses of administration of the drug. The smallest dose that depressed the leukocyte level below 4,000 cells per cubic millimeter was 1 mg. per kilogram daily for 2 days. One patient out of 5 who received 5 mg. per kilogram as a single dose had no hematologic depression. Typical hematologic responses are illustrated in Figs. 1 and 2.

It is a common practice to hospitalize patients for treatment with nitrogen mustard since one cannot predict beforehand which patients will become severely ill with nausea and vomiting despite sedation and antiemetic therapy. Since epoxypropidine, in our experience, caused no more than mild nausea with occasional and transient emesis, it was our practice not to administer any sedatives. Thus, when epoxypropidine was given, hospitalization could be avoided. However, it does not appear that this compound has any therapeutic advantage over nitrogen mustard or other alkylating agents available for intravenous administration.

Certain implications of this work are of more than academic interest. Epoxypropidine fulfilled the requirements for biologic activity, stability of configuration, and solubility. Although it has the same number of reactive groups as diepoxypropylpiperazine, as Gerzon predicted, the increased basicity of epoxypropidine rendered it approximately ten times as active on a weight-for-weight basis. Despite the fact that these theoretical considerations were admirably fulfilled by epoxypropidine, its therapeutic index and apparently its range of activity were not greater than those of the piperazine epoxide. The effect on malignant cells of all clinically useful alkylating agents is limited by the concomitant effect on the bone marrow; all alkylating agents share virtually the same spectrum of activity with dose, rate of administration, and unique side effects notwithstanding. One may, therefore, question to what degree tumor specificity of a drug may be obtained when the pharmacologic basis of its therapeutic effect rests on no consistent qualitative difference between the tumor cells and certain vital normal tissues also characterized by rapid new cell formation (bone marrow, intestinal epithelium).

We wish to thank Eli Lilly & Company, Indianapolis, Indiana, which supplied the epoxypropidine used in this study.

References

1. Burchenal, J. H., Holmberg, E., Wiegand, L., Hemphill, S., and Krakoff, I. H.: The Effects of Epoxypropyl Piperazine Derivatives on a Spectrum of Transplanted Mouse Tumors, Acta Unio internat. contra cancrum. (In press.)
2. Gerzon, K., Cochran, J. E., Jr., White, L. A., Monahan, R., Krumkalns, E. V., Scroggs, R. E., and Mills, J.: Structure-Activity Relationship of Some Diamine Bis-epoxides in Mouse Leukemia, J. Medicinal & Pharm. Chem. 1:223-240, 1959.
3. Johnson, I. S., and Wright, H. F.: Therapeutic Activity of Basic Bis-epoxides in Murine Leukemia and Other Transplantable Mouse Neoplasms, Acta Unio internat. contra cancrum. (In press.)
4. Karnofsky, D. A., and Burchenal, J. H.: Clinical Evaluation of Chemotherapeutic Agents in Cancer. In MacLeod, C. M.: Evaluation of Chemotherapeutic Agents, New York, 1949, Columbia University Press, pp. 191-205.
5. Karnofsky, D. A., editor: Comparative Clinical and Biological Effects of Alkylating Agents, Ann. New York Acad. Sc. **68** (Art. 3):657-1266, 1958.
6. Krakoff, I. H., Miller, D. G., Karnofsky, D. A., Diamond, H. D., Burchenal, J. H., and Craver, L. F.: Clinical Observations of 1,4-Bis (2,3 Epoxypropyl)-Piperazine. One of a New Class of Alkylating Agents, Acta Unio internat. contra cancrum. (In press.)
7. Miller, D. G., Diamond, H. D., and Craver, L. F.: The Clinical Use of Epoxypiperazine, a New Alkylating Agent, in the Treatment of Lymphomas and Other Neoplasms, Cancer Res. (In press.)
8. Ross, W. C. J.: The Reactions of Certain Epoxides in Aqueous Solutions, J. Chem. Soc. London 3:2257-2272, 1950.
9. Stacey, K. A., Cobb, N., Cousen, S. F., and Alexander, P.: The Reaction of the "Radiomimetic" Alkylating Agents With Macromolecules In Vitro, Ann. New York Acad. Sc. **68**:682-701, 1958.
10. Stenderup, A., Bichel, J., and Jensen, K. G.: Effect of Isomers of 1:2, 3:4-Diepoxybutane on Malignant Disorders of the Blood, Proc. VIth Congress of the European Society of Haematology, Basel, 1958, S. Karger AG, pp. 259-261.

Pharmacologic effects of (2-(2,6-dimethylphenoxy) propyl) trimethyl ammonium chloride hydrate in hypertensive patients

The effect of SKF 6890 given intravenously was studied in 10 normotensive subjects and 10 hypertensive patients. It was found that both groups of patients were responsive and an effective dose was 2.5 mg. per kilogram. The drug blocks levarterenol effects (adrenergic blockade). Bioassay studies revealed that the average effective oral dose was around 800 mg.

The compound depresses glomerular filtration rate and renal blood flow when the blood pressure decreases and this is followed by a slight increase of these parameters at the end of the second and third hours. The urinary volume and the sodium and potassium excretion are reduced as the glomerular filtration rate decreases. In long-term experiments, it appears that patients develop tolerance to SKF 6890. After 6 weeks, less than one-fourth of the patients continue to be responsive. The pharmacological observations indicate, however, that this is a new and effective antihypertensive agent which should be used as the basis for synthesizing new compounds of this type.

Joseph B. Rosenfeld, M.D., Jane Straughn, M.D., and John H. Moyer, M.D. *Philadelphia, Pa.*

Hypertension-Renal Unit, Department of Medicine, Hahnemann Medical College and Hospital

It is generally accepted that sympathetic nervous system activity contributes to the blood pressure elevation in patients with essential hypertension. In the past decade different techniques, i.e., sympathectomy, centrally acting sympathetic depressant drugs, ganglion blocking agents and adrenergic blocking drugs, have been used in an attempt to interrupt the sympathetic nervous system pathways which contribute to the increase in blood pressure. The results, limitations, and side effects of these therapies have resulted in a continuous search for more potent and safer compounds. In that it has been reported to block the formation or release of catechol amines at the ends of the postganglionic fibers,[1] a new synthetic quaternary nitrogen compound, (2-(2, 6-dimethylphenoxy) propyl) trimethyl ammonium chloride hydrate (SKF 6890*) may have a different mechanism of action from that of the usual adrenergic blocking agents which block the effects of catechol amines on the receptor sites. Therefore, a clinical study seemed indi-

*SKF 6890 was supplied by Smith Kline & French Laboratories, Philadelphia, Pa.

cated. The objectives were to evaluate the hypotensive effects of SKF 6890 as follows:

1. The effect of the drug given intravenously to hypertensive and normotensive patients.
2. The adrenergic blocking properties of the compound.
3. The optimal oral dose and time response curve of the drug.
4. The effects on renal hemodynamics.
5. The response to long-term administration to ambulatory patients with hypertension.

Materials and methods

Blood pressure response to SKF 6890 given intravenously. Ten normotensive patients not suffering from cardiovascular disease and 10 hypertensive patients (blood pressure greater than 150/100 mm.Hg) were selected at random from the Hypertension Clinic. In the first 3 experiments (normotensive subjects), the SKF 6890 was diluted in 250 ml. of glucose and given in a dosage of 2.5 mg. per Kg. In the next 13 experiments (7 normotensive subjects and 6 hypertensive patients) the dose used was 3 mg. per Kg. and in the last 4 experiments (hypertensive patients), the dose was 4 mg. per Kg. The infusion was given over a period of 15 to 25 minutes and blood pressure readings were taken every 5 minutes during the first half hour, every 15 minutes during the next 2 hours, and every half hour during the last 2 hours of observation. After the infusion was discontinued, blood pressure readings were taken in both the lying and standing positions. The patients were monitored with electrocardiograms.

Observations on the adrenergic blocking effect of SKF 6890. In 3 hypertensive patients 2.5 mg. per Kg. of SKF 6890 was diluted in 10 ml. of 5 per cent glucose and was given intravenously. The effect on blood pressure was then observed. When the blood pressure returned to the control value, an infusion of levarterenol was started (1 mg. in 200 ml. of 5 per cent glucose at a rate of 45 to 50 drops per minute). When the systolic blood pressure increased more than 30 mm.Hg. and the diastolic more than 10 to 15 mm.Hg., SKF 6890 was again given intravenously and the blood pressure taken every minute for the first 30 minutes. After the levarterenol was discontinued and the blood pressure returned to the control values, the same dose of the SKF 6890 was repeated.

Observations of the response to the drug given orally. The smallest effective oral dose was determined by giving the amount of the drug which produced a response when given intravenously. Increasingly larger doses were then given every second day until a reduction in blood pressure was observed. The blood pressure was taken with the patient lying and standing for 12 consecutive hours. When the patients complained of significant side effects, the subsequent dose was not increased.

Observations on renal hemodynamics. Two and one-half milligrams per kilogram of SKF 6890 was diluted in 10 ml. of 5 per cent glucose and was administered slowly (3 to 5 minutes) to 7 hypertensive patients as a single intravenous injection for observations on renal hemodynamics. Consecutive 10 minute collection periods were maintained during a 30 minute control period and for the next 3 hours after the administration of the drug, during which time the effects of blood pressure reduction on water and electrolyte excretion were studied. Glomerular filtration rate was determined by inulin clearance and renal plasma flow by para-aminohippurate clearance. Sodium and potassium excretion rates were determined with the use of a Beckman flame photometer for the analyses. Details of the techniques and analytical procedures have been previously reported by Moyer and Mills.[2,3]

Observations following chronic oral administration for 6 weeks or more. Fifteen patients suffering from essential hypertension and 2 normotensive subjects were randomly selected. They were given SKF 6890 orally for 6 weeks and the dose was increased progressively (titrated). The patients had been carefully examined before

Table I. *Effects of SKF 6890 on mean blood pressure in normotensive and hypertensive patients*

Time (minutes)	Normotensive subjects						Hypertensive patients					
	Supine mean blood pressure*			Upright mean blood pressure*			Supine mean blood pressure*			Upright mean blood pressure*		
	Average 10 patients	% of control	P value less than	Average 10 patients	% of control	P value less than	Average 10 patients	% of control	P value less than	Average 10 patients	% of control	P value less than
Control	101			101			142			137		
0–15	103	102	NS†	—	—		128	90	.02	—	—	
15–30	102	101	NS	89	88	.01	127	89	.001	107	78	.001
60	96	95	.10	86	85	.001	123	87	.001	111	81	.001
90	96	95	NS	79	78	.001	121	85	.02	97	71	.001
120	95	94	NS	83	82	.001	128	90	.20	101	74	.02
150	93	92	.10	80	79	.001	130	91	.20	108	79	.001
180	100	99	NS	90	89	.02	123	87	.05	101	74	.01
240	112	111	NS	97	96	.01	122	86	.05	106	77	.05

*Mean blood pressure = diastolic plus 1/3 the pulse pressure.
†NS, P more than .20.

treatment was started and they were given a placebo for at least 3 weeks. They returned to the Clinic at least once a week and the blood pressure and other signs and symptoms were duly recorded. SKF 6890 was given in a dose range of 500 to 600 mg. daily as a single dose.

Results

Blood pressure response to SKF 6890 given intravenously. The results with SKF 6890 in normotensive subjects are summarized in Table I. Following the intravenous administration of 2.5 to 3.0 mg. per Kg. of the drug, a reduction in the mean blood pressure was observed in all the cases. The maximal response was observed 90 minutes after administration at which time there was a mean reduction of 5 mm. Hg in the supine position and 22 mm. Hg in the upright position (101 to 79 mm. Hg). At the end of 4 hours, a slight rebound phenomenon of the mean blood pressure was noted in the supine position but not in the upright position. There was not a substantial difference in response when the larger (3.0 mg. per Kg. dose) rather than the smaller (2.5 mg. per Kg.) dose was used. A slight increase in blood pressure was noted in most of the cases in the first half hour after administration followed by a substantial reduction which was most marked on standing. The initial rise in blood pressure was frequently accompanied by an increase in the pulse rate of 70 to 100 beats per minute (increasing from 70-80 to 120-150) which lasted 8 to 10 minutes. At that time 2 of the patients complained of retrosternal pressure and shortness of breath. By the time the blood pressure began to drop, most of the patients became relaxed and sleepy. One patient complained of dizziness 2 hours after administration of the drug but the mean blood pressure in the supine position was only 4 mm. Hg below the control value of 110. In the upright position, it had decreased from 110 mm. Hg to 97. The dizziness probably did not result from the reduction in blood pressure but more likely was a direct effect of the drug.

The effect of SKF 6890 when given intravenously to hypertensive patients is summarized in Table I. A dose of 4.0 mg. per Kg. was given to 4 patients but because of side effects, i.e., vomiting, severe nausea, and shortness of breath, the dose was subsequently reduced to 3.0 mg. per Kg. The maximal effect was observed 90 minutes

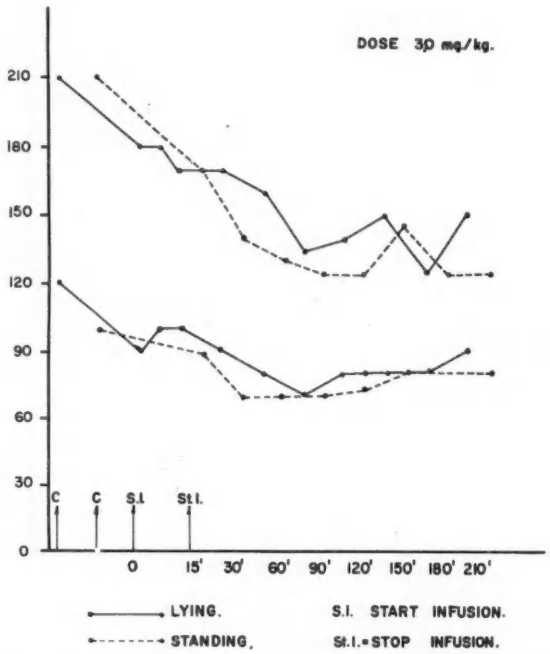


Fig. 1. The effect of SKF 6890 on the blood pressure in a hypertensive patient. The drug was given by the intravenous route in a dose of 3 mg. per Kg. There was a somewhat greater effect in the upright position but the difference in response between the upright and supine positions was minimal in this patient. The response lasted more than 4 hours.

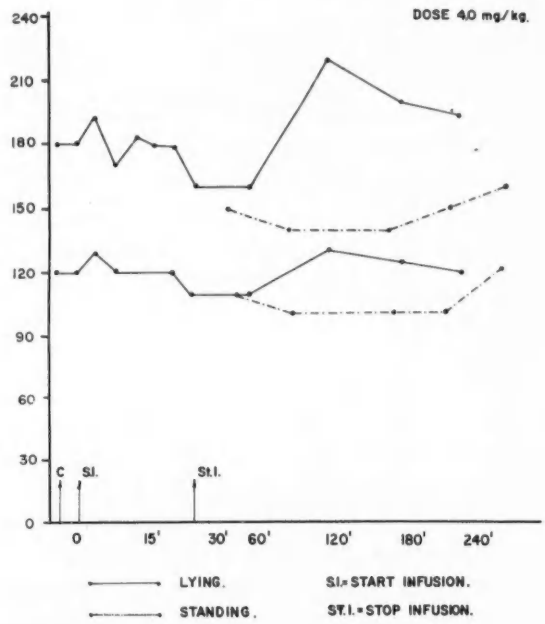


Fig. 2. The effect of SKF 6890 on the blood pressure in a hypertensive patient. There was minimal reduction in both the systolic and diastolic blood pressure when the patient was in the supine position followed by a significant rise in blood pressure above the control level approximately 2 hours after drug administration. When the patient stood up, however, there was a marked orthostatic response during the hyptertensive rebound period.

after the administration of the drug; 4.0 mg. per Kg. did not elicit a greater response than 3.0 mg. per Kg. The mean value for the mean blood pressure of the 10 patients at the time of maximal response (90 minutes) was about 20 mm. Hg lower than the control mean blood pressure in the supine position (142 reduced to 121 mm. Hg) and 40 mm. Hg on standing. At the end of the 4 hour period of observation, the mean blood pressure was still lower than that in the control period by 20 mm. Hg in the supine position and 31 mm. Hg when standing. The patient in Fig. 1 is presented as an example of the group of 6 patients in whom, both supine and standing, there was a sustained reduction in the blood pressure for more than 4 hours following an infusion of SKF 6890 in a dose of 3.0 mg. per Kg. In 2 patients (Fig. 2) there was a drop in the blood pressure while erect, but only a slight drop when supine, followed by substantial and sustained increase in the systolic blood pressure.

In one patient with malignant hypertension who was treated by an infusion of SKF 6890 (4.0 mg. per Kg.) every 6 hours for 3 consecutive days, the response was greatly reduced when the infusion was given at less than 8 hour intervals. The impression that divided doses are of no greater benefit than single doses every 24 hours was also established.

Observations on the adrenergic blocking effect of the SKF 6890. In 3 patients (Fig. 3 is an example), following an intravenous infusion of 2.5 mg. per Kg. of SKF 6890, a drop of 40 mm. Hg or more in the systolic blood pressure was noted. When the blood pressure returned to the control values, it was increased as previously described by an intravenous infusion of levarterenol. Then

the same dose of SKF 6890 was repeated but was divided into two equal doses in 2 patients (for safety purposes) and into two doses of two-thirds and one-third in the third patient. A reduction of more than 50 mm. Hg in the systolic and 25 mm. Hg in the diastolic pressure was noted in the first 2 patients. In Case 23 (Fig. 3), when the 100 mg. dose was administered, the systolic blood pressure reduction was 80 mm. Hg and the diastolic was about 50 mm. Hg. When only one-half of the previous amount was given, the systolic blood pressure reduction was 60 mm. Hg and more time was needed to elicit this response. When the levarterenol was stopped, the blood pressure returned to the control values in 5 minutes. The ability of SKF 6890 to block the hypertensive effect of norepinephrine was clearly demonstrated in these 3 hypertensive patients. It is quite obvious that in man this agent has adrenergic blocking properties by contrast to previous reports of its effect in animals.[1]

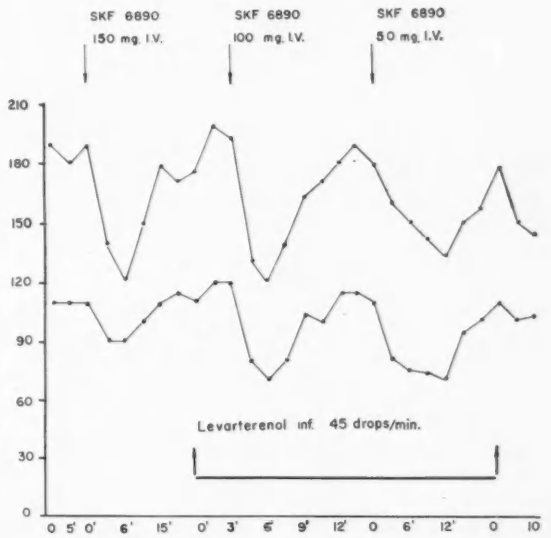


Fig. 3. The effect of SKF 6890 given intravenously before and during the infusion of levarterenol. Here again there appeared to be clear evidence of adrenergic blockade and the response to SKF 6890 showed an even greater reduction in blood pressure when levarterenol was being infused as compared to the response prior to the infusion. This occurred with a dose of 100 mg. given during the infusion as compared with 150 mg. given prior to the infusion of levarterenol. When a dose of SKF 6890 of 50 mg. was given, the response was somewhat delayed and less dramatic.

Observations of the response to the drug given orally. The effective oral dose was determined by titration in 3 hypertensive patients. In the first patient (Fig. 4) the first dose to produce an effect by the oral route was 400 mg. The maximal response was observed in the fifth hour with a fall of 40 mm.Hg in the systolic blood pressure, the effect lasting 2 hours, and was followed by an overshooting phenomenon; the blood pressure rose 60 mm.Hg above the lowest value and by the tenth hour had returned to the control value. When 800 mg. was given, there was a fall of 20 mm.Hg in the first hour in the supine position and a fall of 40 mm. in the standing position. We noted the same overshooting phenomenon with the

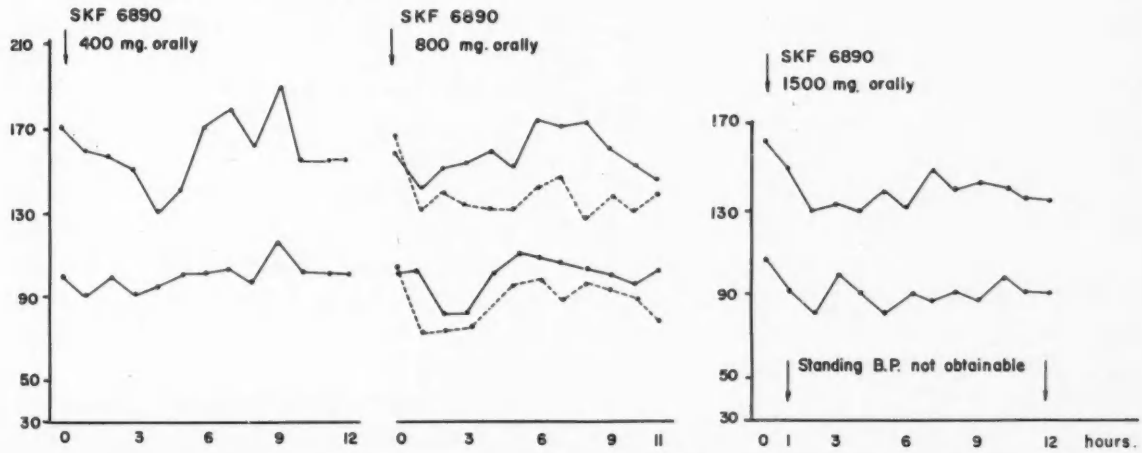


Fig. 4. Blood pressure response to increasing doses of SKF 6890 given by the oral route. As the dose is increased, the orthostatic effect on the blood pressure becomes more pronounced.

Table II. *Renal hemodynamic response to SKF 6890 when given in a single intravenous injection in 7 patients*

Time (minutes)	*Mean blood pressure (mm. Hg)*			*Renal blood flow* (ml. per minute)*			*Renal vascular resistance†*		
	Mean	*% control*	*P value <§*	*Mean*	*% control*	*P value <§*	*Mean*	*% control*	*P value <§*
Control	121	—	—	785	—	—	.16	—	—
0–20	104	85	.05	571	72	.001	.23	140	.05
20–40	114	95	NS‖	687	87	.05	.18	110	.10
60	114	95	NS	677	86	.10	.18	115	NS
120	115	96	NS	763	97	NS	.16	99	NS
180	118	98	NS	921	117	NS	.13	80	NS

*Renal blood flow = $\frac{\text{renal plasma flow}}{1 - \text{hematocrit}}$.

†Renal vascular resistance = $\frac{\text{mean blood pressure}}{\text{renal blood flow}}$.

Table III. *Effect of a single intravenous injection of SKF 6890 on water and electroylte excretion in 7 patients*

Time (minutes)	*Urine volume (ml. per minute)*			*Sodium excretion (μEq. per minute)*		
	Mean	*% control*	*P value <**	*Mean*	*% control*	*P value <**
Control	7.2	—	—	253	—	—
0–20	3.9	54	.05	153	60	NS†
20–40	2.5	35	.02	119	47	.10
60	1.8	25	.01	138	54	.10
120	2.4	33	.02	157	62	.10
180	3.4	47	.01	159	63	NS

*$t = \bar{x} \sqrt{\frac{n\,(n-1)}{S_x\,2}}$ using C (control) as the reference point.

†NS, P value >0.10.

patient in the supine position in the fifth hour and then a return to the control level by the tenth hour, but on standing the hypotensive effect was maintained for the 12 hours of observation. When 1,500 mg. was given, a fall in the systolic pressure of 35 mm.Hg was noted in the second hour with the patient lying down, and was maintained throughout the 12 hours of observation. We were unable to take blood pressure readings with the patient standing because of the excessive reduction in blood pressure and the patient's becoming dizzy on each trial.

The second patient when given 200 mg. had a slight fall in blood pressure by the sixth hour, which was more pronounced on standing. When 300 mg. was administered, the maximal fall was noted by the fifth hour and the reduction was maintained for 4 hours, then the same overshooting appeared. On a 600 mg. dosage, the hypotensive effect (fall of 30 mm.Hg) was observed during the fourth hour and on 1,200 mg. a fall of 60 mm.Hg was noted by the third hour, which was maintained for an additional 3 hours and then the same overshooting appeared.

The third patient appeared to be resistant to the drug and a dose as high as 1,600 mg. did not affect the blood pressure significantly. In all 3 patients higher doses were

Table II
Continued

Glomerular filtration rate (ml. per minute)			*Renal plasma flow (ml. per minute)*			*Filtration fraction‡*		
Mean	*% control*	*P value <§*	*Mean*	*% control*	*P value <§*	*Mean*	*% control*	*P value <§*
58	—	—	461	—	—	.13	—	—
35	60	.001	342	74	.001	.12	86	.02
45	76	.001	407	88	.001	.11	84	NS
55	95	.10	402	87	.10	.15	108	NS
59	101	NS	460	99	NS	.13	98	NS
59	101	NS	499	108	SN	.13	98	NS

‡Filtration fraction = $\frac{\text{glomerular filtration rate}}{\text{renal plasma flow}}$. §$t = \bar{x}\sqrt{\frac{n\,(n-1)}{S_x\,2}}$ using C (control) as the reference point.
‖NS, P value >0.10.

Table III
Continued

Potassium excretion (μEq. per minute)			*Plasma sodium (mEq. per liter)*			*Plasma potassium (mEq. per liter)*		
Mean	*% control*	*P value <**	*Mean*	*% control*	*P value <**	*Mean*	*% control*	*P value <**
44	—	—	143	—	—	3.7	—	—
33	75	NS	141	99	NS	3.6	97	NS
36	82	.10	143	100	NS	3.7	100	NS
33	75	.05	145	101	NS	3.8	103	NS
38	86	NS	146	102	NS	3.9	105	NS
39	88	NS	144	101	NS	3.8	102	NS

not given because of tachycardia, nausea, dizziness, and severe headache.

Observations on renal hemodynamics. The effects on renal hemodynamics, and on water and electrolyte excretion of a single injection of SKF 6890 (2.5 mg. per Kg.) are summarized in Tables II and III.

Within 15 to 20 minutes following the injection, a fall of 17 mm.Hg ($P < .05$) in the mean blood pressure (85 per cent of the control) was noted. This hypotensive effect was followed by a slight and gradual increase in the blood pressure.

As the blood pressure decreased, there was a significant reduction in glomerular filtration rate within the first 40 minutes to 60 per cent and 76 per cent of the control value, respectively. At the end of the first hour, the glomerular filtration rate returned to the initial value. Associated with the reduction in glomerular filtration rate, there was a similar reduction in the renal blood flow which was greatest during the first 20 minutes to 72 per cent of the control rate, remaining depressed during the first hour, and returning to the initial value in the second hour. In the third hour there was a 17 per cent increase in renal blood flow above the control observations.

The reduction of glomerular filtration rate was associated with an increase in the renal vascular resistance to 140 per cent of the control in the first 20 minutes and 115 per cent at the end of the first hour, associated with a slight reduction in the filtration fraction.

These changes in the renal hemodynamics were associated with a striking decrease in urinary volume reaching a maximum at the end of the first hour (25 per cent of the control). There was also a reduction in the sodium and potassium excretion which was maximal in the first 40 minutes, 47 per cent and 75 per cent of the control, respectively. These patients did not show significant changes in the plasma electrolyte concentrations.

Observations following oral administration for 6 weeks or more. Fifteen hypertensive patients and 2 normotensive patients received SKF 6890 for at least 6 weeks. The drug did not affect the blood pressure of the normotensive subjects. Three hypertensive subjects (20 per cent) had a significant* response and 2 of them became normotensive. When the blood pressure was taken with the patient in the upright position, 4 (26 per cent) experienced a significant response and 2 of them became normotensive. When the individual blood pressure responses were evaluated, there was some evidence of tolerance having occurred.

Discussion

SKF 6890 proved to have a hypotensive effect in the normotensive as well as in the hypertensive subject, especially pronounced in the standing position. In the normotensive group, the fall in mean blood pressure while standing was 22 mm. Hg and, in the hypertensive patients, the fall in mean blood pressure was 40 mm. Hg. In the supine position, the normotensive individuals had a very slight reduction in mean blood pressure (5 mm. Hg) but the hypertensive group had a substantial fall (31 mm. Hg), indicating that the drug is more effective in hypertensive patients than in normotensive subjects. Doses above 4.0 mg. per Kg. proved to have many side effects and it was found that the most practical dosage was 2.5 mg. per Kg. Larger doses did not produce a significantly greater effect.

The reduction in blood pressure which was observed during an infusion of norepinephrine proved that this compound has pronounced adrenergic blocking qualities, and if it acts as claimed, by preventing the release or formation of catechol amines,[1] it also blocks the effect of norepinephrine (catechol amines) at the receptor site of the nerve endings.

The bioassay studies revealed that the smallest effective oral dose should be around 600 to 800 mg., which is more than 5 times the minimal effective dose following the intravenous route of administration. At this dose, the maximal effect was observed between the third and fifth hours and the hypotensive effect was more pronounced in the standing position.

Twenty minutes after the intravenous administration of SKF 6890, there was a substantial reduction in the glomerular filtration rate and renal blood flow, associated with reduction in the urine volume, and sodium and potassium excretion. One hour later, there was a gradual return to control values and, at the end of the 3 hours of observation, the glomerular filtration rate and the renal blood flow were increased above the control level, but the urine flow and the sodium and potassium excretion were still depressed. This response is similar to that observed with blood pressure reduction by the administration of ganglionic blocking agents.[2,3]

The long-term study revealed that after a short period of time (1 week to 10 days), when the drug was given orally, most of the patients developed tolerance, so that at the end of the 6 week period only 20 per cent were responsive in the supine position and 27 per cent on standing. No serious side effects were observed and the main complaints were nausea and dizziness. Doses of SKF 6890 greater than 800 mg. given daily produced headache, tachycardia, and vomiting.

*A response was accepted as *significant* when the mean blood pressure was reduced by more than 20 mm. Hg or the blood pressure was reduced to normotensive levels.

References

1. Geus, R. J., McLean, R. A., Pasternack, J., Mattis, P. A., and Ullyot, G. E.: Pharmacology of Trimethyl-(2,6-xylyloxy)-propyl)-Ammonium Chloride, Monohydrate: SKF No. 6890-A or BTM 10, Federation Proc. **18**:1554, 1959.
2. Moyer, J. H., and Mills, L. C.: Hexamethonium —Its Effect on Glomerular Filtration Rate, Maximum Tubular Function and Renal Excretion of Electrolytes, J. Clin. Invest. **32**:172-184, 1953.
3. Mills, L. C., and Moyer, J. H.: The Acute Effects of Hexamethonium on Renal Hemodynamics in Normotensive and Hypertensive Subjects, Am. J. M. Sc. **226**:1-15, 1953.
4. Ford, Ralph V., Spurr, Charles L., and Moyer, John H.: The Problem of Bioassay and Comparative Potency of Diuretics: 1. Bioassay of Mercurial Diuretics, Antibiotic Med. **4**:708-724, 1957.

But no one is under the illusion that nature has so far been vanquished; few dare to hope that she will ever be completely under man's subjection. There are the elements, which seem to mock at all human control: the earth, which quakes, is rent asunder, and buries man and all his works; the water, which in tumult floods and submerges all things; the storm, which drives all before it; there are the diseases, which we have only lately recognized as the attacks of other living creatures; and finally there is the painful riddle of death, for which no remedy at all has yet been found, nor probably ever will be. With these forces nature rises up before us, sublime, pitiless, inexorable; thus she again brings to mind our weakness and helplessness, of which we thought the work of civilization had rid us. It is one of the few noble and gratifying spectacles that men can offer, when in the face of an elemental catastrophe they awake from their muddle and confusion, forget all their internal difficulties and animosities, and remember the great common task, the preservation of mankind against the supremacy of nature.

FROM "FUTURE OF AN ILLUSION" BY SIGMUND FREUD, PAGE 23
BY PERMISSION OF LIVERIGHT, PUBLISHERS, NEW YORK.

Renal effects of hydrochlorothiazide in normal and toxemic pregnancy

The effect of hydrochlorothiazide was studied in 10 hospitalized patients (7 with pre-eclampsia and 3 with normal pregnancy) and 48 ambulatory subjects (30 with normal pregnancy and 18 with toxemia of pregnancy). Hydrochlorothiazide increased the excretion of Na^+, Cl^-, K^+, and water without any significant change in renal plasma flow or glomerular filtration rate. The increase in electrolyte excretion was more marked in the patients with toxemia of pregnancy than in those with normal pregnancy. On the basis of electrolyte excretion hydrochlorothiazide is approximately 10 times as potent as chlorothiazide in pregnant women. As in the case of chlorothiazide, the drug is more effective when edema is present.

N. S. Assali, M.D. *Los Angeles, Calif.*
With the technical assistance of M. Ross and L. Friedman
Department of Obstetrics and Gynecology, University of California Medical Center

Chlorothiazide, given orally or parenterally, significantly increases the renal excretion of sodium, chloride, and water.[1,2,3] These properties make it suitable for the treatment of the edematous state. In fact, patients with edema of diverse etiology have responded to treatment with chlorothiazide.[4,5] Antihypertensive properties have also been described after administration of the drug alone or in combination with vasodepressor drugs.[6] Hydrochlorothiazide, a derivative of chlorothiazide, has been stated to be 10 to 15 times as active as chlorothiazide itself.[7,8,9] These properties make the drug suitable for the treatment of edema. The present report deals with the renal effects of hydrochlorothiazide in normal and toxemic pregnancies.

Material and methods

A. Metabolic studies were carried out in 10 patients, of whom 7 had pre-eclampsia with 3 to 4 plus edema and 3 had normal pregnancy with 1 to 2 plus edema. Patients varied in age from 18 to 38 years; pregnancy varied in duration from 28 to 40 weeks. All the patients were at bed rest in the hospital without any specific medication throughout the periods of study. Each patient received a daily diet containing 85 mEq. of sodium (as sodium chloride).

The studies comprised 3 consecutive periods. The first period, lasting for 3 days, served as a control. During the second period, which lasted from 4 to 5 days and followed immediately after the first, hydrochlorothiazide* tablets were administered

This investigation was supported by grants from the United States Public Health Services.

*Esidrix (Ciba Pharmaceutical Products, Inc.) and HydroDiuril (Merck Sharp & Dohme).

in total daily doses varying from 100 to 150 mg., in 2 to 3 doses. The third period serving as a posttreatment control, lasted for 3 days.

During all periods, the patient's weight, fluid intake and output were recorded daily. Blood pressure and pulse rate while the patient was recumbent in bed were recorded 4 times a day by the same observer. Twenty-four-hour urine specimens preserved with toluene were used for electrolyte determinations.

In 3 patients with pre-eclampsia, renal plasma flow and glomerular filtration rate (inulin and para-aminohippurate clearances) were determined, once in the control period and once on the second or third day of the treatment period. Blood and urine electrolytes as well as inulin and para-aminohippuric acid were determined by methods currently used in our laboratories.[3]

B. Clinical studies were carried out on 30 subjects with normal pregnancy who had from 1 to 2 plus edema and 18 patients classified as having a mild form of toxemia of pregnancy. All were ambulatory and were observed in the prenatal clinics for a period of over 2 months.

Each normal pregnant patient was given from 100 to 150 mg. of hydrochlorothiazide per day in 2 to 3 doses. The drug was administered in periods of 5 to 6 days alternated with 2 day drug-free periods. The patients with toxemia of pregnancy received the same dose of the drug for the entire period of observation without interruption. No other medication was administered to any of these patients during the periods of study. A diet containing approximately 2 Gm. of sodium chloride per day was allowed. Blood pressure, pulse rate, and weight were recorded once every week by the same observer, always at the same time, and under the same environmental conditions.

Results

Systemic effects. All patients tolerated hydrochlorothiazide well. A few instances of mild nausea occurred, but these could not be attributed specifically to the drug. The patients with toxemia of pregnancy who were treated while hospitalized had an average fall in blood pressure of 25 mm.Hg systolic and 15 mm.Hg diastolic. It was difficult to be certain, however, whether this fall was due to the action of the drug or to bed rest. The blood pressure of the ambulatory patients did not change significantly.

Effects on blood and urine electrolytes. Table I presents data on blood and urine composition in a typical patient with normal pregnancy and similar data from a representative case of toxemia of pregnancy. Table II lists the average values of electrolyte excretion in all the patients studied.

In the control period, normal pregnant subjects had a stable sodium and chloride excretion in relation to the intake. During the administration of hydrochlorothiazide, the output of these ions increased significantly (Tables I and II). The increment was higher during the first 2 days of treatment than in the last days. In the posttreatment period, the excretion of sodium and chloride decreased to control levels or even below. The excretion rate of potassium rose during the treatment period, but less than that of Na and Cl. The increased excretion of electrolytes was accompanied by diuresis and loss of weight. Despite the marked increase in electrolyte excretion, plasma electrolytes remained practically unchanged.

In the patients with toxemia of pregnancy, the excretion of sodium and chloride in the control period was also fairly stable in relation to intake (Tables I, *B*, and II). On the first day of treatment, the excretion of these ions nearly tripled. Although on subsequent treatment days the excretion of Na and Cl decreased somewhat, it was still significantly higher than in any control day. In the posttreatment period the excretion of Na and Cl reverted to pretreatment levels. The excretion rate of potassium also increased, but significantly less than that of sodium and chloride. The loss of electrolytes was accompanied by an increase in urine output and loss of weight which were significantly greater than those in the nor-

Table I. *Intake and output, urine and plasma electrolytes during control, treatment, and posttreatment periods*

Period	Day	Intake (ml./day)	Output (ml./day)	Weight (Kg.)	Urine			Plasma		
					Na (mEq./day)	Cl (mEq./day)	K (mEq./day)	Na (mEq./L.)	Cl (mEq./L.)	K (mEq./L.)
A. Patient M. S., normal pregnancy, 1 plus edema										
Control	1	2,200	1,900	56.4	75	60	24	135	110	4.0
	2	2,420	2,200	56.0	89	72	22	138	108	4.5
	3	2,520	2,430	56.0	82	70	20	137	112	4.2
Hydrochlorothiazide 100 mg. daily	4	2,250	2,300	55.5	110	98	38	136	110	4.0
	5	2,100	2,250	55.2	115	104	30	135	109	4.4
	6	1,950	1,975	55.0	95	78	28	134	108	3.8
	7	2,050	2,235	54.5	105	85	22	136	110	3.7
Posttreatment	8	2,150	1,800	55.0	65	48	22	136	110	4.2
	9	2,175	1,950	55.1	80	72	27	138	112	4.5
	10	2,100	2,025	55.4	72	60	28	138	110	4.3
B. Patient L. S., pre-eclampsia, 4 plus edema										
Control	1	3,250	3,100	75.0	85	72	32	142	110	4.5
	2	3,100	3,010	75.1	78	62	28	140	112	4.2
	3	3,050	2,970	75.0	80	75	24	139	112	4.0
Hydrochlorothiazide 150 mg. daily	4	3,050	3,200	74.0	210	180	42	139	110	4.0
	5	3,100	3,350	74.1	175	145	32	138	108	3.8
	6	3,010	3,275	73.2	195	160	30	140	109	3.9
	7	3,200	3,260	73.0	100	82	28	138	110	4.2
	8	3,025	3,115	72.8	110	95	27	—	—	—
Posttreatment	9	2,910	2,900	73.1	65	52	18	142	110	4.3
	10	2,875	2,700	73.2	48	40	16	140	108	4.2
	11	2,850	2,690	73.4	75	65	20	140	112	4.5

Table II. *Data on the excretion of Na, Cl, and K in the patients with normal pregnancy and in those with toxemia of pregnancy. Each value represents the average for the control, treatment, and posttreatment periods*

Patient	Diagnosis	Daily dose (mg.)	Na			Cl			K		
			Control	Treatment	Post-treatment	Control	Treatment	Post-treatment	Control	Treatment	Post-treatment
1	Normal	100	82	106	72	67	91	60	22	30	26
2	Normal	150	87	132	71	75	125	62	28	36	24
3	Normal	150	78	112	68	68	102	58	24	38	23
1	Toxemia	100	81	158	62	70	132	52	28	32	18
2	Toxemia	100	89	178	75	75	162	61	32	42	30
3	Toxemia	100	79	168	70	69	151	60	38	48	36
4	Toxemia	150	92	195	80	80	178	72	30	40	28
5	Toxemia	150	80	160	65	68	145	50	24	36	20
6	Toxemia	150	90	175	86	75	132	60	22	31	16
7	Toxemia	150	78	162	71	65	145	58	26	38	20

mally pregnant subjects. Despite the marked saluretic effect, plasma Na^+, Cl^-, and K^+ did not change significantly.

Effects on renal hemodynamics. Hydrochlorothiazide given orally in the dose of 100 to 150 mg. per day did not significantly alter renal plasma flow or glomerular filtration rate (Table III).

Clinical results. The 30 normally pregnant subjects who were followed for more than 2 months lost weight while they were receiving the drug. The magnitude of weight loss varied from one patient to another and depended a great deal on the amount of edema which was present prior to the beginning of treatment. During the first 2 weeks of treatment, the maximum weight loss observed in this group was 6 pounds and the minimum was less than 1 pound. Despite the continuation of treatment, as patients lost their edema, the effect of the drug decreased.

The patients with toxemia of pregnancy also lost weight throughout the treatment but the weight loss was significantly greater than that of the normally pregnant patients. The maximum individual weekly loss during the first 2 weeks was 12 pounds and the minimum was 2 pounds. Here again, the weight loss decreased as the patients became edema free.

Table III. *Effects of hydrochlorothiazide on urine flow, glomerular filtration rate (GFR), and renal plasma flow (RPF). All figures were corrected for body surface area*

Patient	Period	U.F. (ml./min.)	GFR (ml./min.)	RPF (ml./min.)
1	Control	2.5	105	575
	Treatment	3.6	102	565
2	Control	2.8	112	615
	Treatment	4.1	106	620
3	Control	1.7	98	535
	Treatment	2.6	102	545

Discussion

Hydrochlorothiazide has a diuretic action similar to that of chlorothiazide. An increase in electrolyte excretion, particularly sodium and chloride, invariably follows its administration.

Although no comparison of the dose response of the two drugs was made in this study, the magnitude of electrolyte excretion after the administration of 100 to 150 mg. of hydrochlorothiazide was approximately equal to that observed by us in the same type of patients after 1,000 to 1,500 mg. of chlorothiazide.[3] These findings are

in agreement with those obtained by others in animals and man.[7,8,9] Hydrochlorothiazide is, therefore, approximately 10 times as potent as chlorothiazide in the pregnant woman.

As in the case of chlorothiazide, the diuretic effects of hydrochlorothiazide are more marked and more lasting in the toxemic, edematous patients than in the majority of normal subjects, who have less edema. When edema of a significant degree was present in a normally pregnant subject, the effects of the drug were more intense. In addition, the diuretic action was always more pronounced at the beginning of the treatment than at the end, and, as the treatment progressed and the patients lost their edema, the diuretic action of the drug decreased. This provides a safety valve against severe electrolyte depletion since, when the patient loses excess fluid and electrolytes provided by edema, further administration of the drug tends to have little effect.

References

1. Boyer, Karl H., Baer, John E., Russo, Horace F., and Maimbach, Audrey: Chlorothiazide (6-chloro-7-sulfamyl-1, 2, 4-benzothiadiazine-1, 1-dioxide): The Enhancement of Sodium Chloride Excretion, Federation Proc. **16**:278, 1957.
2. Moyer, J. H., Ford, R. V., and Spurr, C. L.: Pharmacodynamics of Chlorothiazide (Diuril), an Orally Effective Non-mercurial Diuretic Agent, Proc. Soc. Exper. Biol. & Med. **95**:529, 1957.
3. Assali, N. S., Judd, L., Mondz, N., and Dasgupta, K.: Diuretic Effects of Chlorothiazide in Toxemia of Pregnancy, J. Lab. & Clin. Med. **52**:423, 1958.
4. Schreiner, G. E., and Bloomer, H. A.: Effect of Chlorothiazide on the Edema of Cirrhosis, Nephrosis, Congestive Heart Failure and Chronic Renal Insufficiency, New England J. Med. **257**:1016, 1957.
5. Assali, N. S., Judd, L., and Mondz, N.: Clinical and Metabolic Study of Chlorothiazide (Diuril), J.A.M.A. **169**:26, 1959.
6. Wilkins, R. W.: New Drugs for Hypertension, With Special Reference to Chlorothiazide, New England J. Med. **257**:1026, 1957.
7. Richterich, R., Spring, P., and Thönen, H.: Klinische Erfahrungen mit Hydrochlorothiazid, einem neuen, oral verabreichbaren Sulfonamid-diureticum, Schweiz. med Wchnschr. **89**:353, 1959.
8. Bartorelli, C., Gargano, N., and Zanchetti, A.: Étude comparative de l'action sali-diurétique de l'hydro-chlorothiazide et du chlorothiazide, Schweiz. med. Wchnschr. **89**:331, 1959.
9. Moyer, J. H., Fuchs, M., Irie, S., and Bodi, T.: Some Observations on the Pharmacology of Hydrochlorothiazide, Am. J. Cardiol. **3**:113, 1959.

Safe new drugs and their control under law

It has been our aim to review briefly Food and Drug Administration's methods in evaluating new drugs, and it is our hope that this will provide a better understanding of the role and function of the Food and Drug Administration in its regulation of new drugs. This will, in turn, assist physicians to meet their responsibilities in this important field.

William H. Kessenich, M.D. *Washington, D.C.*
Medical Director, Food and Drug Administration, Department of Health, Education and Welfare

When the question is asked, "Aren't drugs safer now than they used to be?" the answer is very often heard, "Well, the ads say they are, and, besides, they have to be safe or they couldn't be on the market."

It is incumbent upon physicians who use these drugs to know that safety as employed here is a relative term based on certain conditions of use. To prescribe the newly developed drugs intelligently they should be aware of the methods used in developing the drug for the market and the means by which the designation "safe" came to be applied to any individual pharmaceutical.

There can be little doubt that the tremendous strides of the pharmaceutical industry in the United States over the past twenty years have resulted in the greatest development of new drugs ever experienced in any like period of recorded medical history. The fact that, of every 100 prescriptions written, 90 are for drugs that were not available even fifteen years ago is just one indication of the impact on medical practice that these developments have wrought. Only a very superficial knowledge of the economic picture of our country is necessary to understand that the gross sales of individual companies in the pharmaceutical industry have skyrocketed in these recent years with a corresponding rise in the value of their stocks. For the investors in these companies this growth has been an exciting and rewarding story, but beyond economic prosperity what else does this growth mean to the physicians, pharmacists, and to the rest of the population, many of whom within the next year will have to depend upon one of these new drugs for their well-being? It means even more new drugs will be available. Of the total income that accrues to the pharmaceutical industry, there is a substantial portion which each year is designated for research. It is the boast of the pharmaceutical industry that the percentage of total sales returned to research and development is the highest in any field of business. As a result of their phenomenal growth, the amount of money that has been spent for research has increased year by year until it is estimated that, in 1960, the industry's

research budget will be something over 190 million dollars. This, coupled with Government-financed research in amounts of about 300 million dollars, can only result in even more numerous and more potent drugs being developed and made available for use.

These figures give some insight into the impact on medical practice that the drug boom has caused. Looking at it in another light, in the past year there were between 45 and 50 new single medicinal chemical entities introduced and a total of from 375 to 400 new pharmaceutical specialty products. It can reasonably be expected that these numbers will continue to spiral upward. This places a tremendous burden on the prescribing physican who hardly has time to learn the coined names of the newly introduced drugs, much less to know which of them are simply duplications. And the responsibility to his patient is greater than ever, since before he writes a prescription calling for a new drug he should know enough about the drug to recognize the possible contraindications and side effects which may occur and also know what precautions must be exercised in using these potent therapeutic agents.

Results have confirmed the fact that the drugs developed in this era are powerful agents for good. The physician can easily and successfully attack and overcome, with the aid of these new drugs, diseases which until now have not yielded successfully to treatment. But the blessing has been mixed, for with the increased potency in combating disease, there is similarily an increase in the undesirable effects. One hospital study recently was reported as showing that 5 per cent of 1,000 consecutive admissions to a major hospital in New York City were due to side effects of drug therapy, which leads us to repeat the question, "Are drugs safe?" Safety must be considered largely from a relative point of view. When the possible benefit is weighed against the possible harm of new drugs that have reached the market the scale will tip easily in favor of the beneficial effect they have—provided the balance on which they are measured is the sound professional judgment of a well-informed practitioner. The physician is recognized as having the responsibility for using drugs safely. Allied with the physician stands an arm of the Federal Government which also is charged with the responsibility of assuring that new drugs are safe before they can be marketed and promoted to the physician for his prescription. This unit is the Food and Drug Administration and its authority for regulating new drugs is contained in the new drug section of the Federal Food, Drug, and Cosmetic Act.

Until the passage of the present Federal Food, Drug, and Cosmetic Act in 1938, there had been no restrictions on the introduction and interstate marketing of new, untested, and possibly dangerous drugs. It took a tragic incident to bring about legislation to require adequate testing of drugs for safety before they could be marketed.

What has come to be known as the "Elixir of Sulfanilamide Tragedy" resulted in more than a hundred deaths. The sulfanilamide disaster of 1937 resulted from marketing a diethylene glycol solution of sulfanilamide without investigation of the toxicity of the solvent. There had been a problem with the solubility of sulfanilamide in ordinary and known safe solvents so without even rudimentary toxicologic evaluation the diethylene glycol was used and the product was marketed. It was not long before a series of deaths reported from across the country brought the inspectional forces of the Food and Drug Administration into play. The investigations soon established the guilt of the deadly Elixir of Sulfanilamide. The product was removed from the market quickly, but the only charge that could be made against the drug at that time was that it was misbranded since it was labeled an "elixir" and, in fact, failed to meet the definition that an elixir is an alcoholic solution.

With the unhappy story of this event fresh in its memory, the Congress passed new Food and Drug legislation in 1938, leg-

islation which had failed to pass in several previous years and legislation which now contained provisions that all "new drugs" must be shown to be "safe" before marketing. This "new drug" provision was intended to insure that tests would be completed before introduction of a new drug into interstate commerce. It was to prohibit such introduction until the manufacturer had submitted full information showing that the drug had been adequately tested and not found to be unsafe for use under the conditions prescribed in the labeling. The mandate from Congress further indicated that this not be a licensing provision but be intended merely to prevent immature marketing of new drugs not properly tested for safety.

The report accompanying the bill also pointed out that the new-drug provision did not put the Federal Government into the business of developing new drugs nor did it require the Government to duplicate laboratory and clinical tests made by responsible manufacturers. The provision merely set up a method for the authoritative review of the manufacturers' tests which would not unreasonably delay the entry of new drugs into the market. It requires the manufacturer to submit a new drug application to the Government for review of safety studies before he can vend the products of his research and development.

Let us now examine how this application with its mass of data is reviewed and evaluated by the experts of the New Drug Branch, a section of the Food and Drug Administration's Bureau of Medicine.

What is a new drug under the law? When must a new product be subjected to this safety evaluation?

The definition of a new drug is contained in the Federal Food, Drug, and Cosmetic Act. A new drug is defined as one which, when used as directed in its labeling, is not generally recognized as safe by experts qualified by scientific training and experience to evaluate the safety of drugs. It is pointed out by regulation that a product may be a new drug not only on account of its active ingredient, but also because of any new solvent, excipient, coating, or other component. A combination of two old drugs may be a "new drug" or even a combination of drugs of recognized safety may be made a "new drug" by a change in proportion of its ingredients. A drug which is recognized as safe for the treatment or prevention of one disease may become a new drug when recommended for treatment of another disease or for its effects on another structure or function of the body. Newness of a drug may stem from a change in dosage or in method or duration of administration from those generally recognized as safe. The second part of the definition states that a new drug continues to be a new drug even after it has been shown to be safe by investigations. This status holds until it has been used to a material extent or for a material time under the conditions of use which have been provided for in an application.

As a consequence of this section of the Federal law, anyone who wishes legally to distribute in interstate commerce a product which falls within the legal definition of a "new drug," as stated above, must first obtain an effective new drug application. The only exceptions involve a group of 5 antibiotics and their derivatives which by law must be certified, and several biologic preparations, such as toxins, antitoxins, vaccines, and certain blood products which are subject to license control by the Public Health Service. In order to obtain an effective application, the applicant must conduct such studies as are reasonably applicable to demonstrate his product's safety and then submit all of his data to the Food and Drug Administration.

The application, together with a completed application form (see Fig. 1), must be submitted and must contain among other information:

1. Full reports of all investigations that have been made to show whether or not the drug is safe for use.

2. A full list of the articles used as components of the drug.

Form FD-356—Rev. 1956
DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE
Food and Drug Administration

Form Approved
Budget Bureau No. 57-R003.1
Expiration Date: 12-31-62

Original ☐ or Supplemental ☐ Application

Name of applicant.....

Address.....

Date.....

Name of new drug.....

(If this is a supplemental application, see Item (8))

To the SECRETARY OF HEALTH, EDUCATION, AND WELFARE
For the Commissioner of Food and Drugs
Washington 25, D. C.

DEAR SIR:

The undersigned, ..., submits this application with respect to a new drug pursuant to section 505 (b) of the Federal Food, Drug, and Cosmetic Act. Attached hereto, in duplicate, and constituting a part of this application are the following:

(1) Full reports of all investigations that have been made to show whether or not the drug is safe for use.

(a) An application may be incomplete or may be refused unless it includes full reports of adequate tests by all methods reasonably applicable to show whether or not the drug is safe for use as suggested in the proposed labeling. The reports ordinarily should include detailed data derived from appropriate animal or other biological experiments in which the methods used and the results obtained are clearly set forth. Reports of all clinical tests by experts, qualified by scientific training and experience to evaluate the safety of drugs, should be attached and ordinarily should include detailed information pertaining to each individual treated, including age, sex, conditions treated, dosage, frequency of administration, duration of administration of the drug, results of clinical and laboratory examinations made, and a full statement of any adverse effects and therapeutic results observed.

(b) The complete composition and/or method of manufacture of the new drug used in each submitted report of investigation should be shown to the extent necessary to establish its identity, strength, quality, and purity if it differs from the description in parts (2), (3), or (4) of the application in any way that would bias an evaluation of the report.

(c) The unexplained omission of any reports of investigations made with the drug by the applicant or submitted to him by an investigator he supplied with the drug that would bias an evaluation of the safety of the drug constitutes grounds for the refusal or suspension of an application.

(2) A full list of the articles used as components of the drug. (This list should include all substances used in the synthesis, extraction, or other method of preparation of any new-drug substance, regardless of whether they undergo chemical change in the process. Each substance should be identified by its common English name or complete chemical name, using structural formulas when necessary for specific identification. If any proprietary preparation is used as a component, the proprietary name should be followed by a complete quantitative statement of composition. Reasonable alternatives for any listed substance may be specified.)

(3) A full statement of the composition of the drug. (This statement should set forth the name and amount of each ingredient, whether active or not, contained in a stated quantity of the drug in the form in which it is to be distributed; as, for example, amount per tablet or per milliliter, in addition to a representative batch formula. Any calculated excess of an ingredient over the label declaration should be designated as such and percent excess shown. Reasonable variations may be specified.)

(4) (a) A full description of the methods used in the manufacture, processing, and packing of the drug. Included in this description should be full information on the following, in sufficient detail to permit evaluation of the adequacy of the manufacturing, processing, and packing methods to determine and preserve the identity, strength, quality, and purity of the drug:

(i) The methods used in the synthesis, extraction, isolation, or purification of any new-drug substance. When the specifications and controls applied to such substance (described in part (4) (b) of this form) are inadequate in themselves to determine its identity, strength, quality, and purity, the methods should be described in sufficient detail, including quantities used, times, temperature, pH, solvents, etc., to determine these characteristics. Alternative methods or variations in methods within reasonable limits that do not affect such characteristics of the substance may be specified.

(ii) The methods used in processing and packing each proposed dosage form of the new drug, including a description of the container or other packaging material.

(iii) If the applicant does not himself perform all the manufacturing, processing, and packing operations for any new-drug substance or the new drug, his statement identifying each person who will perform a part of such operations and designating the part and a signed statement from each such person, fully describing the methods he uses directly or by reference.

(b) A full description of the facilities and controls used for the manufacture, processing, and packing of the drug.

(Included in this description should be full information on the following in sufficient detail to permit evaluation of the adequacy of the described methods, facilities, and controls to preserve the identity, strength, quality, and purity of the drug.)

(i) A description of the physical facilities including plant and equipment used in manufacturing, processing, packing, and control operations on the new drug.

(ii) If the applicant does not himself perform all the manufacturing, processing, packing, and control operations, his statement identifying each person who will perform a part of such operations and designating the part; and a signed statement from each such person fully describing the facilities and controls he uses in his part of the operations directly or by reference.

(iii) Precautions to insure proper identity, strength, quality, and purity of the raw materials, whether active or not, including the specifications for acceptance of each lot of raw material.

(iv) Whether or not each lot of raw materials is given a serial number to identify it, and the use made of such numbers in subsequent plant operations.

(v) Method of preparation of formula card, and manner in which it is used.

(vi) Number of individuals checking weight or volume of each individual ingredient entering into each batch of the drug.

(vii) Whether or not the total weight or volume of each batch is determined at any stage of the manufacturing process subsequent to making up a batch according to the formula card and at what stage and by whom it is done.

(viii) Precautions to check the actual packaged yield produced from a batch of the drug with the theoretical yield.

(ix) Precautions to insure that the proper labels are placed on the drug for a particular lot, including provisions for label storage and inventory control.

Fig. 1.

3. A full statement of the composition of the drug.

4. A full description of the methods used in and the facilities and controls used for the manufacture, processing, and packing of the drug.

5. Samples of the finished product.

6. Copies of all labeling to be used for the drug.

These applications, depending on the drug, vary in size from one small volume to a submission consisting of multiple volumes amounting to thousands of pages and close to 100 pounds of material. They are received and reviewed by the New Drug Branch. The relatively small professional staff of this Branch, consisting of physicians, chemists, and pharmacists, must bear

(x) The analytical controls used during the various stages of the manufacturing, processing, and packing of the drug, including a detailed description of the collection of samples and the analytical procedures to which they are subjected. If the article is one which is represented to be sterile, the same information should be given for sterility controls. Include the standards required for acceptance of each lot of the finished drug.

(xi) An explanation of the exact significance of any batch control numbers used in the manufacturing, processing, and packing of the drug, including any such control numbers that may appear on the label of the finished article. State whether or not any of the numbers appear on invoices and describe any other methods used to permit determination of the distribution of any batch if its recall is required.

(xii) A complete description of and the data derived from studies of the stability of the drug. If the data indicate that an expiration date is needed to preserve the identity, strength, quality, and purity of the drug until it is used, a statement of an expiration date.

(xiii) Additional procedures employed which are designed to prevent contamination and otherwise insure proper control of the product.

(5) Three finished market packages of the drug, and other samples of the drug or its components on request.

(When finished market packages of the drug are not available to submit with the application, state that finished market packages conforming to the description under part (4) (a) (ii) and labeled as provided in part (6) of the application will be submitted as soon as available and prior to the marketing of the drug. In case the drug is available only in limited quantity, state the extent to which samples of the drug and its components will be available on request.)

(6) Five copies of each label and other labeling to be used for the drug.

(a) Each label, or other labeling, should be clearly identified to show its position on, or the manner in which it accompanies, the market package.

(b) The labeling on or within the retail package should include adequate directions for use by the layman under all the conditions for which the drug is intended for lay use, or is to be prescribed, recommended, or suggested in any labeling or advertising sponsored by or on behalf of the applicant and directed to laymen.

(c) The labeling on or within the retail package, or a brochure or other printed matter specifically identified on such label or labeling and made available to practitioners, should contain adequate information for use of the drug by such practitioners under all the conditions for which the drug is intended or is to be prescribed, recommended, or suggested in any labeling or advertising sponsored by or on behalf of the applicant.

(d) Labeling bearing adequate information for use of the drug by practitioners should be a part of the retail package of injections and any other drug that may be unsafe for the intended use unless such information is immediately available to the practitioner.

(e) Typewritten or other draft labeling copy may be accepted for conditional consideration of an application, provided a statement is made that final printed labeling identical in content to the draft copy provided for in the application will be submitted as soon as available and prior to the marketing of the drug.

(7) State whether the drug is (or is not) limited in its labeling and by this application to use under the professional supervision of a practitioner licensed by law to administer it.

(8) If this is a supplemental application, full information on each proposed change concerning any statement made in the effective application.

(After an application is effective, a supplemental application may propose changes. The supplemental application may omit statements made in the effective application concerning which no change is proposed. A supplemental application should be submitted for any change beyond the variations provided for in the application, that may alter the conditions of use, the labeling, the safety, identity, strength, quality, or purity of the drug or the adequacy of manufacturing methods, facilities, or controls to preserve them. When necessary for the safety of the drug, a supplemental application may be required to specify a period of time within which the proposed change will be made; and in such case the distribution of the drug after such time without such change constitutes distribution without an effective new-drug application. A supplemental application is not required when the article is no longer a new drug unless the proposed change itself causes it to become a new drug. If a material change is made from the representations in an effective application for a new drug before a supplement is effective for such change, the application may be suspended under § 130.27.)

(9) It is understood that all representations in this application regarding the components, composition, manufacturing methods, facilities, controls, and labeling apply to the drug produced until an effective supplement to the application provides for a change or the article is no longer a new drug.

Very truly yours,

(Applicant)

Per ---

(Indicate authority)

This application must be signed by the applicant or by an authorized attorney, agent, or official.

The data specified under the several numbered headings should be on separate sheets or sets of sheets, suitably identified. The sample of the drug, if sent under separate cover, should be addressed to the New Drug Branch and identified on the outside of the shipping package with the name of the applicant and the name of the drug as shown on the application.

The applicant will be notified of the date on which his application is filed. An incomplete application, or one which has not been submitted in duplicate, will usually be retained but not filed as an application provided for in section 505 (b) of the act. The applicant will be notified in what respect his application is incomplete.

ALL APPLICATIONS AND CORRESPONDENCE SHOULD BE SUBMITTED IN DUPLICATE

Fig. 1. *Cont'd*

the burden of evaluating the completeness and adequacy of the studies that have been devised, conducted, and reported by these applicants. The review procedure calls for the assistance of other Food and Drug Administration technical divisions whenever indicated. In some instances the advice of original investigators or experts outside the Food and Drug Administration may be sought. Let us note some of the considerations and problems that face the evaluators in their processing of new drug applications.

The investigations that are submitted ordinarily are expected to include full reports of adequate tests by all methods reasonably applicable and should contain detailed data derived from appropriate animal or other biologic experiments in which the methods used and the results obtained are clearly set

forth. Reports of all clinical tests by experts qualified by scientific training and experience to evaluate the safety of drugs should be attached and should include detailed information pertaining to each individual treated, including age, sex, conditions treated, dosage and frequency of administration, duration of administration of the drug, results of clinical and laboratory examinations made, and a full statement of any adverse effects and therapeutic results observed. The kind and amount of information that is indicated will depend on several factors, such as the nature of the drug and its indication, and must be determined individually for each new drug. While it is not possible to make dogmatic statements concerning the requirements with respect to the animal toxicity studies, the members of the Division of Pharmacology of the Food and Drug Adminstration have collaborated in preparing a guide outlining the elements of a program for the appraisal of the safety of chemicals in foods, drugs, and cosmetics.

In a general way we expect the animal studies to include an acute toxicity experiment (LD_{50}'s) in at least two or three species by oral and intravenous administration. There should also be longer-term animal studies, depending on the clinical uses for which the drug will be recommended. If it is a drug which will probably be used only once or for only a day or 2, then a 2 to 3 week subacute study should usually suffice. If on the other hand the intended use of the drug will usually require prolonged and continuous administration, then chronic experiments of from 6 months to a year are probably indicated. Customarily these experiments are carried out by giving the drug in animal food. If the product is an injectable, a toxicity study should be included, the drug being used by the route of administration that will be recommended clinically. It is recommended that a rodent and a nonrodent species be used in these toxicity studies so usually rats and dogs are selected. The drug should be administered to three groups of animals each at a different dosage level and these compared with a control group. The lowest level should be at a high therapeutic dose and the highest at a dose that will produce toxic effects. Blood and urine studies should be performed routinely and periodically during the course of the experiment and other laboratory procedures that are appropriate should be included. At the termination of the study the animals should be sacrificed and the organs studied for gross and microscopic lesions.

Certain varieties of drugs, such as topical preparations, will require special methods of study. Techniques have been devised which will test the potentiality of such preparations to produce primary irritation, sensitization, or systemic effect due to absorption from the skin. An injectable drug should be studied also for its local irritating effect.

When this amount of animal information has been acquired and studied, it is usually sufficient to indicate progress to careful administration of the drug to humans. In the process of acquiring the toxicity information, there has also been in most instances careful study of the pharmacologic activity and of the metabolic fate of the drug. These studies will have provided information relative to the principal sites of activity of the drug, will indicate a range of dosage which can be expected to produce an effect, and will suggest potential side effects as well as the resultant effect that might be encountered in event of overdosage. Armed with these facts the clinical phase of the investigations proceeds.

While the guides to the type of animal study that is usually accepted cannot be precisely spelled out, they are still much more definitive than are the criteria that can be established for the clinical studies. In using drugs on human subjects it is obviously impossible to specify such a definite program of drug use as we can in the rodents and dogs where an LD_{50} or subacute study at a toxic dose level is suggested. The possibility of unanticipated effects of a certain drug must be considered

and this tangent explored. It is not possible to state simply how many patients must be studied to demonstrate the safety of a drug. A series of clinical groups are often required, with each group being studied for some different aspect of the agent and all of these facets must be accumulated and fitted together before the picture of the drug becomes clear. The investigators, clinical facilities, and the patient material will vary. Because of these and many, many other variables, it quickly can be recognized that to forecast what will be an adequate program of clinical study is virtually impossible. And it is for these same reasons that our most difficult decisions in evaluating the adequacy of evidence of safety come in the review of the clinical data.

Who performs these clinical investigations? The requirement is that the investigations be carried out by experts qualified by training and experience to evaluate the safety of drugs. The Food and Drug Administration has zealously refrained from ever attempting to exert any control or regulation over the practice of medicine and in accord with this policy has never defined any professional requirements which must be met by those who study investigational drugs and whose reports are submitted in new drug applications. To a considerable extent it is the quality of the study which determines the weight it will carry in the evaluation of a drug. It is only natural, however, that, other things being equal, we would lean heavily on studies performed by recognized and acknowledged specialists and by recognized medical colleges and research facilities.

It is also to be expected, if the drug under study is a new anesthetic, that the more critical evaluation will come from qualified anesthesiologists or if a drug is to be offered for the treatment of diabetes that it should be studied by specialists in that field. If the drug is to be offered for pediatric use as well as for adults, then the clinical studies should include groups of children. This does not mean to say that a certain amount of very valuable and helpful information might not also be obtained by extending the studies and having some investigations done by competent general practitioners. One can hardly expect one or even two studies to provide all of the information about the clinical use of a drug. It is important that the clinical evaluation be done by several competent investigators and preferably by investigators from a variety of clinical centers. The reasons for these considerations should be obvious. It is difficult at best to get an accurate picture of the clinical behavior of a drug. We must consider that one evaluator may be enthusiastic about *any* new drug, and we likewise recognize that other clinicians are hypercritical of any new agent. There must be an adequate number of studies then to balance these extremes and reach a fair and accurate judgment.

There are times when, as a result of the information gleaned from the animal studies, we know that special laboratory studies are indicated. Some of these may be relatively routine and capable of being performed anywhere, but on the other hand there are drugs which require that more elaborate and specialized tests be performed, facilities for which are available in only a limited number of clinical centers. All of these factors add up to the conclusion that an adequate clinical investigative program must entail, at times, multiple studies on a multitude of patients and employ a variety of scientific disciplines. As we mentioned previously, some of the most difficult decisions that the Food and Drug evaluators must make involve these clinical studies.

After these brief thoughts on what a new drug application should include, here are some of the problems and difficulties that are actually encountered in new drug submissions. The most frequently encountered deficiency is in reports that are sketchy and incomplete. Investigators, before undertaking a new drug study, are obligated to complete an investigator's form and should therefore know that careful observation of their patients is indi-

cated. They should know, too, that these studies usually include information on age, sex, conditions treated, dosage, and frequency of administration, duration of administration, and details of indicated laboratory studies. Having this information, the problem is usually that of recording and reporting it. Some studies are reported only in the testimonial form, such as "I used your drug on many patients. It was successful in the majority and there were no significant side effects." This is of no value whatsoever. It is impossible to know any of the facts from this type of report. We do not know what side effects were looked for or what in this individual's opinion is considered significant. Did this include laboratory studies and what were the details? A report that "lab studies were within normal limits" fails again to tell us which tests were done and what is considered normal. It is impossible for the pharmaceutical firm to devise adequate labeling for their drug based on such reports and they are equally worthless to the physician at the Food and Drug Administration who must review the report.

The applicant who submits a new drug application—generally this is a pharmaceutical firm—is responsible for obtaining and submitting these reports from busy practitioners. These men are often eager to do the work on the new drug, but the mechanical chore of writing the report which is time consuming and often tedious is the problem. Many firms have facilitated the return of complete and full reports by devising forms which require only short fill-in answers or check forms to get most of the necessary information. Even with such short-cut methods for obtaining information, there still should be a general appraisal of the drug by the investigator; this is not to be confused with the testimonial which was criticized above. When an investigator accompanies the detailed case reports with an evaluation of the new drug based on his experience and in comparison with older and recognized agents of similar type, it is of help in reaching a decision.

All of the animal and clinical studies having been considered, labeling is the next step in the new drug evaluation. The labeling of a drug includes not only the label on the article and its container, but also all the information accompanying the article. If the proposed drug is to be sold without prescription, then the labeling must contain adequate directions for use by the laity. If, as is usually the case, the drug is intended for prescription use, the labeling should contain adequate information for use of the drug by physicians. This means that the container labels, as well as the package insert or the brochure intended for the physician, are part of the information which is subjected to critical processing in the new drug application. This labeling, then, is another vital part of the consideration of whether the new drug is "safe," for it is based on the information thus presented. A drug should be safe for use as labeled and it is in this labeling that the physician is given the information he needs to use the drug properly. It is in the labeling—the label *and* the brochure—that the physician is told of the indications, the dosage, the contraindications, the precautions he must observe, and the side effects he might expect. This information is the culmination of all the information obtained from the animal experimentation and the actual clinical trials.

One of *our* major problems is trying to see that the presentation of the information in the brochures is an accurate and unslanted appraisal of the facts obtained from these investigations. We must be sure the dosage recommended is that which was used and shown safe in the trials. We must also be sure that favorable studies are not given more prominence than unfavorable studies. Even though a certain adverse effect may have been reported only rarely in the clinical studies and possibly by only one or two investigators, we think that in all probability this effect will be encountered when the drug is marketed. A decision must be made with the recognition that *safety is relative.* No drug is entirely safe.

There are a number of drugs which are marketed and are well known to be definitely hazardous. In this connection consideration is given to the value of a drug as a therapeutic agent. If it is a drug that is lifesaving or if it is a palliative agent in the treatment of a serious disease for which there is no better remedy, then some degree of hazard can be sanctioned. If, on the other hand, the drug is offered for some minor ailment such as a simple headache or a minor cold, then little or no risk should be tolerated. There are all gradations of necessity and safety between these extremes. In processing a new drug application, our considerations are based only on an evaluation of the safety of a drug and we can control claims for effectiveness of the agent only inasmuch as they directly involve safety. As an example, we take the position that safety can be questioned in the extreme situation of an ineffective agent, albeit safe per se, offered as a cure for a potentially fatal illness, especially when there is other therapy of recognized value available.

The brochure for a new drug is frequently the only source of information on the new product available to the physician. It must be reliable. Because of delays in publication, new drugs are very often available to the practitioner before reports of the studies which were the basis for considering them suitable for marketing. The Food and Drug Administration does not have the authority to withhold from the market any drug simply because the data regarding the drug are not published and available to the profession. It is, therefore, our responsibility to assure that the physician's brochure or package insert accurately and sufficiently reflects the known facts about the drug. To accomplish this end often involves the problem of semantics. There are frequent conferences between the new drug applicants and Food and Drug representatives to agree on the language to be used in these brochures which will present the drug to the physician in its proper perspective. In each case, however, it is our policy to require a brochure which gives the whole picture. This brochure is available and usually presents a more nearly complete story than most of the promotional literature which floods the mails.

There are other parts of the review of a new drug application which to the clinician, on the surface, would seem to be of little interest. These involve review of information which, in fact, is of extreme importance to the practitioner in assuring the safety of the drugs he uses. The manufacturer is required to submit information on components, composition, the facilities, and controls that are used in the production of the drug, which will assure uniformity in identity, quality, strength, and purity of the finished drug product.

In this regard each new drug application is required to contain a complete listing of the components of the drug. The list includes not only the ingredients of the final product, but of all substances used in the synthesis or preparation of the drug. Such information may alert us to the possibility of contamination of the final product by some undesirable substance which may have been carried over during manufacture. It may suggest the necessity for control procedures or specifications to insure the absence from the final product of such a substance or its presence in only a limited and tolerable concentration. The full statement of the composition of the drug is the quantitative formula for the dosage form and includes, for example, the materials in the coatings, solvents, and flavorings. A batch formula serves as a check on the quantitative composition and will reveal averages which are used in the manufacturing procedure. If this seems like too much detail, recall that it was an untried solvent in the Elixir of Sulfanilamide that was the toxic substance and which was the stimulus for the new drug portion of the Food and Drug Law.

In order to prevent the wrong material from accidentally getting into a drug, we consider it good manufacturing practice to check each lot of raw materials for accep-

tance with respect to identity, strength, quality, and purity. In addition, analytical controls should be used during various stages of the manufacturing process and are certainly required for the finished product. Such procedures as assay methods and sterility controls are also a part of the information required. In recent years we have been requiring reports of stability studies on new drugs and a statement of an expiration date if needed. This is of particular importance with certain dosage forms, such as parenterals and some liquid preparations.

The description of the manufacturing procedures and controls which have just been mentioned is an important part of the new drug application. Such procedures and controls are necessary in establishing the safety of a drug. They insure that the product manufactured will be of uniform standard. As a result of such controls, the physician has come to expect this standard and confidently presumes that the drugs he uses and prescribes routinely and uniformly meet it.

Completion of a review of a new drug application has included the evaluation of pharmacologists, chemists, experts in the drug manufacturing procedures, and physicians. Within 60 calendar days a decision must be reached. If significant details in any area have been omitted, we may refuse to file the application and the applicant is given the opportunity of collecting the missing details and amending the application. If, in the opinion of the Food and Drug Administration the information is complete, but inadequate to establish safety, the applicant may withdraw his application and proceed to accumulate aditional evidence. If, on the other hand, he considers that he has adequately shown safety, he may be given the opportunity of a hearing before a hearing examiner representing the Secretary of the Department of Health, Education and Welfare, to hear the facts. If the decision of this hearing is adverse to the applicant, he may appeal to the Federal courts. In practice, the scientists representing the applicant and the scientists of the Food and Drug Administration usually can discuss these matters on a very high sicentific plane and resolve their differences satisfactorily. As a result, there is very rarely an occasion when consideration must be given to the administrative hearing or court procedure.

If the Food and Drug Administration is satisfied that adequate study has been performed and reported and that with the indicated pattern of labeling the drug is safe for use, the new drug application is allowed to become effective. This simply means, at this point, that the Government has been satisfied that the drug can be safely used and will interpose no objection to its interstate marketing. As indicated in the legislative history of the enabling statute, this constitutes neither a licensing of the drug nor is it an approval of its claims. Not infrequently we take exception in correspondence with the firms to statements in the labeling which we do not believe to be justified. Our objections may or may not be heeded. As we have stated, we cannot prevent an application from becoming effective on the basis of unsubstantiated claims unless these can be directly related to safety. When a new drug is marketed, it is subject to the other provisions of the Act which include those of misbranding because of false or misleading claims. In regulating drugs under this section of the law, however, unlike the new drug section, the burden of proof in a Federal court is upon the Government. We have learned by experience that proof of misbranding usually requires not only expert opinion, but also negative data obtained by adequate clinical investigation.

A new drug remains a matter of concern to us even after it is the subject of a new drug application and is marketed. Any change or revision of the original application must be covered by supplements which are reviewed and handled just as the original application. These supplements may provide for an additional tablet of different potency from that initially distributed, for

a change in the inactive ingredients, for modification of manufacturing procedures, for distribution of the drug under the label of another firm, or for revised labeling. Revised labeling may relate to a change in dosage, added indications for use, or additional precautions based on further experience with the product. In comparison with about 500 original applications which may be received in a year, there are submitted within the same period 1,000 to 2,500 supplements for applications already effective.

We follow with interest and continuing concern the marketing experience with the drug. Staff limitations prevent us from pursuing this to the extent we consider adequate. We try to cover the current medical literature, but we recognize frequent lags because of publication schedules. We may receive reports from individual physicians, from the Food and Drug Administration field inspectional staff, or from the firm which distributes the drug. Soon we are hopeful of establishing a unit within the Food and Drug Administration whose function will be to obtain as soon as possible information on adverse drug reactions. The reports we receive occasionally justify revised labeling such as dosage reduction or added precautions. This has been done sometimes on our initiative and sometimes on the initiative of the firm. In some cases it has been necessary to remove a drug from the market. Under such circumstances an effective application can be suspended by legal procedure.

How long is a drug considered new and subject to this control? The second part of the definition of a new drug states that it continues to be new even after it has been shown safe by investigations. It is sometimes shown that, in actual use, drugs are not as safe as was predicted on the basis of investigative studies. The new drug status holds until the drug has been used to a substantial extent and for a material time under the conditions of use which have been provided for in the new drug application. This means that, although one firm holds an effective application and is marketing the drug, other firms must obtain their own effective applications before marketing the same drug as long as it has new drug status. Here again, differences of opinion may arise as to whether a drug has been used to a sufficient extent or for a sufficient time to justify its removal from the category of new drugs. Decisions with respect to whether a new product is a new drug requiring a new drug application are of extreme importance to the pharmaceutical firm. The time and expense involved in preparing an application are of considerable commercial significance and we do not have the authority to dictate such decisions. If a difference of opinion does exist which cannot be mutually resolved, the question can be answered in a Federal court.

It has been just over 20 years now that new drugs have been subjected to this Federal control. Over 12,000 new drug applications have been reviewed by the Food and Drug Administration. Working under a strict time limit of 60 to 180 days and with a relatively small staff, the New Drug Branch of the Food and Drug Administration has made and continues to make a significant contribution to the medical profession and to the public health in general.

Briefly, let us consider some of the advantages to the physician that result from governmental review of new drugs. The physician does not have to consider his first trial with a new drug as a novel experiment. He can use new drugs with the confidence and assurance that studies have already been made which have verified the drug as safe. Governmental review has provided labeling which is available to the physician giving him all of the essential information needed by him to use the drug safely.

There are some things which are *not* done with which the physician should be equally familiar in his considerations of new drugs. Food and Drug review does not constitute licensing nor is it an approval of the claims of efficacy. It does not mean, either, that the Food and Drug Administration is dictating how the physician should

practice medicine or what drug is best for any particular patient. It simply implies that the physician has available to him information gained by the experience of his colleagues and reflected in the brochure to use in reaching his decision as to whether or not to use a drug.

Even in view of the Government's role in regulating new drugs, the *physician* still maintains a major share of the responsibility in this matter of developing new drugs and in properly using these potent and rapidly expanding new weapons against disease. The physician, from clinical investigator to general practitioner, can only meet his serious responsibility by having a better insight into and knowledge of the process of development and marketing of new drugs.

The clinical investigator should know what is expected of him when he submits the results of his work and experience to a pharmaceutical firm. He is assuming the responsibility of providing the basis for the information which will allow the drug to be marketed in the first place, as well as the basis for the information which will be disseminated to the medical profession at large, telling them how to use the drug effectively and safely. The clinical investigator does much more than simply satisfy his clinical curiosities or serve as a consultant to a pharmaceutical firm as an aid in maintaining and supporting his clinical facilities. His role and responsibility in new drug development are vital.

The practitioner, too, has a responsibility which he must meet to care for his patients properly. He must know as much about a new drug as possible before he can safely prescribe it. He must know the precautions that must be observed, the side effects, and the contraindications to the use of the drug. He must know where to find this information, and, until the drug has been used to such an extent that his information is available in the literature as review articles or in texts of pharmacology, the official brochure is his best source.

References

Federal Food, Drug, and Cosmetic Act as amended. Washington, D.C., U.S. Department of Health, Education and Welfare; Food and Drug Administration.

New Drug Regulations Under the Federal Food, Drug, and Cosmetic Act, Washington, D.C., U.S. Department of Health, Education and Welfare; Food and Drug Administration.

Dunn, Charles Wesley: Federal Food, Drug, and Cosmetic Act, a Statement of Its Legislative Record. New York, 1938, G. E. Stechert & Company.

Van Winkle, Walton, Jr., Herwick, Robert P., Calvery, Herbert O., and Smith, Austin: Laboratory and Clinical Appraisal of New Drugs, J.A.M.A. **126**:958-961, 1944.

Smith, Ralph G.: Assuring the Safety of New Drugs, Pub. Health Rep. **71**:590-593, 1956.

Modell, Walter, and Houde, Raymond W.: Factors Influencing Clinical Evaluation of Drugs With Special Reference to the Double-Blind Technique, J.A.M.A. **167**:2190-2199, 1958.

Modell, Walter: The Basis for the Choice and Use of New Drugs, GP **20**:129-137, 1958.

Schiffrin, M. J.: Ethics and New Drugs, Postgrad. Med. **24**:305-313, 1958.

Testing New Drugs, Some of the Difficulties, Brit. M. J. **1**:296-298, 1959.

Lehman, Arnold J.: Newer Trends in the Laboratory Evaluation of the Safety of Drugs, Bull. Parenteral Drug A. **13**:1-5, 1959.

Meyers, Earl L.: Stability Tests of Parenterals Under the New Drug Regulations, Food, Drug & Cosmetic Law Journal **14**:514-526, 1959.

Holland, Albert H., Jr.: Drugs, Doctors, and Directives, J. Am. Geriatrics Soc. **7**:148-152, 1959.

Appraisal of the Safety of Chemicals in Foods, Drugs, and Cosmetics, by the Staff of the Division of Pharmacology, Food and Drug Administration, Department of Health, Education and Welfare. Austin, Texas, 1959, Association of Food and Drug Officials of U.S.

Epinephrine and norepinephrine actions and use in man

Our present concepts of the role and use of epinephrine and norepinephrine in man are largely based on the doctrine of epinephrine as the emergency hormone of the adrenal medulla, originally outlined by Cannon and his associates, and the more recent finding that norepinephrine serves as neurotransmitter of the adrenergic nervous system and also as a medullary hormone. Important contributions to the knowledge of these amines have also been gained by empirical work on their use as remedies in various conditions.

While soon after its discovery epinephrine was introduced into the therapeutic arsenal, it was almost 50 years before norepinephrine came to be used more regularly as a drug.

As soon as it became recognized in the 1940's, however, that norepinephrine served as an adrenal medullary hormone as well as adrenergic nerve transmitter, the situation changed. It is true that inadequate experimentation and defective conclusions in the beginning caused some of the important physiologic differences between the two hormones to be overlooked, but gradually the pattern of actions and, based upon these, the indications for the use of both epinephrine and norepinephrine became clearer.

In this review an outline is given of what is now regarded as the physiologic roles of the two hormones, based on a survey of some of their most important pharmacologic actions in man, and these are compared with reported results of the therapeutic effects of the hormones.

U. S. von Euler, M.D. *Stockholm, Sweden*
Karolinska Institutet, Stockholm

On many occasions unexplained or unexpected clinical effects of a drug have induced investigators to reconsider the ideas of its physiologic and pharmacologic actions and to seek new information as to their mechanism. This has also been the case for epinephrine* and norepinephrine.†

Norepinephrine was synthesized as early as 1904 and Biberfeld[7] in Austria advocated its therapeutic use in 1906 on the basis of some of its pharmacologic actions, which in several ways made it a suitable preparation and from some points of view even better than epinephrine. By virtue, however, of its property as a recognized physiologic substance and because of the rather incomplete knowledge of the actions of the two substances, epinephrine alone was accepted as a drug, and no serious efforts were made to find a place for norepinephrine in therapy.

Much of the older literature on the sub-

*Adrenalin.

†Arterenol, levarterenol, Levophed.

ject will be found in the author's[27] monograph "Noradrenaline." The reader is also referred to the more recent, well-documented review of Gaddum and Holzbauer.[40]

Some aspects of the actions of epinephrine in relation to its use as a cardiovascular stimulant

Since the pioneer work of Cannon and his co-workers it seems well established that epinephrine as an emergency hormone plays only a minor role during ordinary life conditions. Removal of the adrenal medulla is not accompanied by any obvious disturbances except in situations of stress of various kinds. On the other hand epinephrine is a powerful stimulant of the heart and the vessels in various vascular areas, an efficient metabolic catalyzer, and an effective inhibitor and excitor of various smooth-muscle organs. Epinephrine, therefore, may be looked upon as a highly active drug but hardly as a substitute during failing physiologic production, owing to the rather special conditions of its natural release. Although the value of epinephrine in some clinical syndromes, as in asthma, is hardly disputed, its preferential use in other conditions has been the subject of controversial opinions. While there is no question about its efficiency as a cardiac stimulant, it is by no means clear that it should be regarded as the drug of choice in combating cardiovascular disturbances. In a careful judgment of the totality of its effects one has to take into account a number of actions other than the direct, stimulating one on the frequency and the force of the heartbeat and the vascular effects. Many reports have dealt with its tendency to "overstimulate" the heart as shown by the appearance of irregularities in the normal activating mechanisms, its tendency to reduce the efficiency of the heart by driving it in an uneconomic way, and its forceful but often unwanted redistribution of the blood. This series of events may be entirely tolerable and indeed even useful in the healthy heart and circulatory system during situations of stress, but it seems rather bold and questionable to assume that it would be equally desirable in attempts to improve the circulation when it shows signs of failure for one reason or another. Even though a word of caution thus seems to be justified regarding the clinical and therapeutic use of adrenaline in circulatory disturbances, there is ample evidence to show that epinephrine may have useful effects, particularly in conditions where the circulatory apparatus is essentially normal and healthy but temporarily depressed.

The important dilatation of the vascular bed in skeletal muscle caused by epinephrine suggests that it serves to improve the functional alertness of the system chiefly involved in locomotion and fight. Is such an effect desirable or does it serve a useful purpose in the clinical treatment of failing circulation, perhaps maintained by a heart working at the very limit of its capacity and using its last resources? An answer in the affirmative seems to require more positive proof than is available at present.

Physiology and therapeutic use of norepinephrine in man

The physiology of norepinephrine is in essence the physiology of the adrenergic nervous system. The heart and practically all vessels supplied with a muscular coat receive sympathetic postsynaptic fibers of the adrenergic type. We are therefore concerned with an almost ubiquitous distribution of this system and its neurotransmitter. Thus far, there is no indication that these fibers release any other transmitter substance than norepinephrine, which of course does not exclude the possibility that stimulation of sympathetic nerves may release small amounts of adrenaline from chromaffin cells, supplied with preganglionic fibers running in these nerves. The amounts of epinephrine released in this way are very small, usually less than 10 per cent of the total catecholamines. Bilateral adrenalectomy in man makes the epinephrine normally excreted in urine fall by more than 80 per cent, reducing the ratio of epineph-

rine to norepinephrine from about 20 per cent to 3 to 5 per cent.[31] After degeneration of the adrenergic nerves to an organ the latter still retains most of its adrenaline,[36,46] which then most likely depends on the presence of the chromaffin cells demonstrated in many organs and tissues.

If we therefore have to admit that norepinephrine is present in the whole of the circulatory system including the heart, and continuously released, as judged by its continuous outflow in urine, we also have to discuss in which ways its actions deviate from those of epinephrine, which may be released into the blood stream during various conditions.

Important investigations on the basic circulatory effects of epinephrine and norepinephrine have been carried out by Goldenberg and associates[45] and by Barcroft and Konzett.[4] The subject has also been reviewed more recently.[29,30]

Heart. The bulk of catecholamines in the heart of mammals consists of norepinephrine which is distributed in the coronary vessels as well as in the myocardium. In addition, small amounts (less than 10 per cent of the total) of epinephrine are present, presumably in chromaffin cells. Thus Paasonen and Krayer[72] found that reserpine treatment in the rat did not deplete epinephrine in the same way as norepinephrine which would be expected if epinephrine was contained in, and released from, adrenergic fibers. It has recently been shown in animals that depletion of myocardial catecholamines results in depression of cardiac contractility and the intrinsic rhythmicity of the ventricle. It seems permissible therefore to conclude that these catecholamines are of physiologic importance for maintaining normal conditions in the heart.[58,78a] This should not be overlooked in reserpine treatment which, even in small doses administered over a prolonged period, may deplete the catecholamines in the heart almost completely.[93]

The effects of epinephrine and norepinephrine on the heart are in many respects similar. Both amines stimulate the frequency and the force of the heartbeat.[42,79] As pointed out by Rushmer and West,[79] the effect of norepinephrine may be masked in vivo by the homeostatic reflex apparatus but will be obvious after exclusion of this, for instance, after ganglionic blocking agents.

The effects of epinephrine and norepinephrine on the efficiency and metabolism of the heart have been the subject of much discussion. Generally speaking epinephrine is a much stronger stimulant of metabolic processes than norepinephrine and should accordingly have a stronger action on oxygen consumption.

It is well known that infusion of epinephrine and norepinephrine in man may cause the appearance of ectopic beats and arrhythmias especially when they are administered in large doses. The actions are of a complicated nature, and, depending on the conditions, epinephrine may either provoke or abolish ectopic arrhythmias even in the same patient.[83]

Influence of catecholamines on the blood flow through various vascular regions. At moderate infusion rates, 0.1 to 0.3 μg per kilogram per minute in man, epinephrine and norepinephrine cause characteristic effects on the flow through various organs, depending on the effects on peripheral resistance and the available pressure head. These effects have been often reviewed and the results will not be repeated in detail here. It seems of interest, however, to draw attention to some implications from the results obtained by infusions of norepinephrine in man and animals.

It is a common finding that norepinephrine may reduce the blood flow in various vascular regions when infused even in moderate doses. This has been described for the kidney, the liver, the brain, and other important regions. These findings would naturally induce some hesitation in the clinician who might wish to use the drug in cases of inadequate circulation. The results in normal subjects give, however, only partial information about the effects which might be expected in a failing circulatory

system. As pointed out by Corcoran, Wagner, and Page,[19] noradrenaline, although it usually causes vasoconstriction in the kidney, has a diuretic effect in hypertensive patients, the filtration rate being unaltered or increased. In those cases in which the filtration rate was decreased, urine volume also decreased. At low blood pressure levels, however, when diuresis was depressed, infusion of norepinephrine causes as a rule an increased urinary flow, indicating that the rise in blood pressure overcompensates the vasoconstriction produced. Only with large doses of norepinephrine may the vasoconstriction be so intense—as with epinephrine—that diuresis suffers. Epinephrine seems to exert a stronger vasoconstrictor influence than norepinephrine on the kidney.[3] Both epinephrine and norepinephrine induce proteinuria regularly in man, suggesting that these hormones play a role in the transient proteinuria of various stressful conditions.[56]

Studies of the effect on the hepatic blood flow of infusions of epinephrine and norepinephrine have not yielded uniform results, the variations probably depending on differences in dose and mode of administration. An increased flow when epinephrine was infused and no change in flow after norepinephrine[5] have been reported in man, when moderate doses were used.

Of special interest are the findings and interpretations of the effects of epinephrine and norepinephrine infusions on the blood flow through the brain. King, Sokoloff, and Wechsler[55] found a slight increase after epinephrine and a slight decrease after norepinephrine, and similar results were noted by Sensenbach, Madison, and Ochs.[84] On the other hand Bovet and associates[12] observed vasoconstriction and decreased flow with both epinephrine and norepinephrine in the dog.

Such effects might call for caution in using norepinephrine infusions in conditions of inadequate blood pressure and blood flow for fear of curtailing cerebral oxygen supply; however, in such conditions the effect may be the opposite, i.e., an increase in flow occurs as a rule, owing to the augmented pressure head which overcomes the moderate vasoconstriction in the cerebral vascular region.

Particularly informative in this respect are the observations in man made by Moyer, Morris, and Snyder.[69] While a continuous infusion of norepinephrine caused a decrease in cerebral flow in normotensive subjects, concomitantly with a rise in blood pressure, the same infusion actually increased the cerebral blood flow when the blood pressure level was lowered by ganglionic blocking agents.

It thus seems that the vasoconstriction and sometimes reduced flow during norepinephrine infusion in normal conditions give no valid information about the effects in circulatory failure when blood pressure is low. Under such circumstances the effect is generally an increase in flow.

Considering the fact that norepinephrine dilates the coronary vessels even in high doses, it is easy to understand that an infusion of norepinephrine would improve the coronary flow any time it is effective in raising the systemic blood pressure. On the other hand it has been demonstrated that epinephrine may constrict the coronary bed,[96] indicating that the increased coronary flow after epinephrine is due to the more forceful heartbeat.

Effect on lung circulation. Infusion of epinephrine and norepinephrine generally causes a rise in pulmonary arterial and wedge pressure in man.[37,74] Similar effects were found on inhalation of epinephrine in normal subjects.[1] These results are in agreement with those observed in the dog.[62]

In this connection it might be recalled that the small pulmonary vessels have a scanty vasomotor supply as judged by the content of norepinephrine, while the large vessels have a rather higher content, suggesting a stronger reaction.[33]

Effect on the veins. A constriction of the total vascular bed results after administration of epinephrine and norepinephrine indicating that they act also on the veins.[44] This effect is of importance in reducing the

capacity of the venous part of the vascular bed and thus aids in improving circulation in conditions where venous pooling occurs, such as in certain states of shock.

The importance of constriction of the venous bed by the vasoconstrictor neurotransmitter and, consequently, by norepinephrine, for maintaining arterial pressure and cardiac output under a hydrostatic load is clearly shown by studies on dogs by Salzman and Leverett.[81]

Metabolic actions. While intravenous infusion of epinephrine causes a considerable rise in blood sugar and in blood lactic acid, and a fall in the inorganic phosphorus, norepinephrine has no or only a slight effect in these respects.[51]

In this connection it seems appropriate to bring up the question as to whether or not the catecholamines cause a release of ACTH. This has been shown for both epinephrine and norepinephrine in the rat. On the other hand, opinions as to the effect of epinephrine and norepinephrine in man differ. Thorn and co-workers[90] found no increase in urinary corticosteroids after infusion of epinephrine in man, but subsequent workers have found evidence for a release of ACTH after infusions of epinephrine. An increased excretion of corticosteroids has also been observed in cases of chromaffin cell tumors which secrete epinephrine but not in those which secrete norepinephrine solely.*

Effects on the nervous system and relationships to psychic activity. The role of epinephrine and norepinephrine in synaptic transmission has been extensively studied by Marrazzi,[66] who found evidence for a reciprocal chemical equilibrium between cholinergic and adrenergic neurohormonal influences. Thus adrenergic compounds were found to be synaptic inhibitors not only in ganglionic transmission but also at cerebral synapses. Norepinephrine was less effective in this respect than epinephrine, and required about fifteen times as big a dose to cause an equal degree of inhibition.

Facilitating effects have, however, been described with smaller doses of catecholamines.[14,64] Reference should also be made to the stimulating effects of catecholamines on the reticular system in the brain stem.[11]

The peculiar effect of epinephrine on the emotional status is well known, and usually manifests itself in a feeling of uneasiness and apprehension, sometimes reaching a state of anxiety or fright. These effects are only occasionally encountered after administration of norepinephrine. Swan[88] noted from his interviews with 60 subjects who received epinephrine by intravenous infusion that the effects were usually more marked than those experienced in previous situations of alarm, and concluded that epinephrine may well enter the circulation during stress, but at rates rather below 10 μg per minute. These findings are in harmony with observations on the excretion in urine during stressful situations, as studied extensively by Elmadjian, Hope, and Lamson[24] and others. From the work of Elmadjian and his co-workers it also became clear that in certain conditions also norepinephrine is released in increased amounts, particularly in subjects who react in a more aggressive way. Similar findings have been reported by Funkenstein, Greenblatt, and Solomon[39] and by Cohen, Silverman, and Zuidema.[18]

The alleged relationships between certain psychic disorders and disturbances of the catecholamine metabolism have attracted much interest in the past few years. It does not seem possible at present to make any definite statements about these relationships, however. A critical survey of this field has recently been made by Kety[54] to which the reader is referred. It should be mentioned, however, that in certain mental disorders typical alterations in the production and release of catecholamines seem to occur.[6] The increased excretion of epinephrine in mania is especially noteworthy.

New interest in the central effects of the catecholamines has been induced by the finding that reserpine depletes the stores of

*Querido, A.: Personal communication, 1955.

these amines present in the brain. On the basis of this interesting finding,[52] a theory of the role of norepinephrine as central neurotransmitter, has been developed.[13]

Some general aspects of the therapeutic use of catecholamines

While the conditions in which the therapeutic use of epinephrine is indicated seem to be fairly well established, the indications for the use of norepinephrine are in some respects controversial.

In the previous section some of the principal actions of norepinephrine, especially on the cardiovascular system, have been briefly enumerated. From these accounts it seems reasonably well founded that norepinephrine is an effective agent in improving the activity of the heart, and, by concomitant peripheral vasoconstriction when the sympathetic activity is diminished, in raising the blood pressure.

One might then ask in which cases of hypotension is a beneficial effect of norepinephrine to be expected? Favorable results have been reported from the treatment of hypotension occurring after various drugs and poisons, including ganglionic blocking agents, reserpine, barbiturates, choline esters, spinal anesthesia, and vagal syncope. This is not surprising since in these cases the failing vasomotor and cardiac nerve activity can be substituted by the natural substance, norepinephrine.

Less clear is the effect of norepinephrine in hemorrhagic or oligemic shock. In these conditions, where loss of fluid is the most important factor, the organism would be expected to respond by strong vasoconstriction and increased activity of the whole sympathoadrenal system. It is therefore of interest that Gilmore[43] has found a significantly increased survival rate in dogs which, immediately after severe hemorrhage, received an infusion of norepinephrine during one hour prior to reinfusion of blood. This effect is probably not so much one of peripheral vasoconstriction but may rather depend on an improvement of the force of the heartbeat, as suggested by numerous reports from animal experiments.[42,57,79,92] During the bled state there might be a diminished reactivity of the cardiovascular system which could be partially compensated for by norepinephrine. A treatment of this kind is obviously of emergency character, pending reinfusion of a suitable fluid, but might represent a stopgap intervention. On the other hand it should be emphasized that too large doses of norepinephrine may actually prove deleterious in such conditions.[15]

In many kinds of toxemias and other cases where the reactivity of the heart and vessels is low, as in prolonged hypoxia and asphyxia, the response to norepinephrine may be low or virtually absent. This may often be the case in severe shock, particularly when the condition has been of some duration.

When, because of an unsatisfactory response, larger doses of norepinephrine are resorted to, it is important to bear in mind the phenomenon of postinfusion hypotension. For this reason the dose level should be kept as low as possible.

A large body of literature has grown up on the therapeutic use of norepinephrine in hypotension and shock. This comprises various common surgical conditions,[20] myocardial infarct,[82] postoperative and infectious shock and drug poisoning,[70,78] anaphylactic and oligemic shock,[68] pulmonary embolism,[60,97] and other conditions.

Postinfusion effects of epinephrine and norepinephrine on blood pressure and blood flow. In considering the effect of epinephrine and norepinephrine on circulation it is of importance to bear in mind an effect which may, to a greater or smaller extent, influence the result of its administration. This is a depressant action on the central and ganglionic transmission which has been observed in a variety of conditions. Particularly noteworthy in this respect are the findings of Swan,[87] who showed that intravenous infusions of epinephrine in man cause a secondary dilatation of the vessels of the normally innervated hand but not in the limb after sym-

pathectomy. He drew the conclusion that this secondary dilatation is a result of central depression of vasomotor tone. An effect which may be of a similar kind is regularly observed upon prolonged intravenous infusion of epinephrine and, although to a lesser degree, also by norepinephrine. After the end of infusion there is sometimes a sharp drop in the blood pressure which may last for as long as one hour and which is accompanied, in animal experiments, by a marked reduction in blood flow through the hind part.[22] This effect is increasingly apparent with increase in dose and is well known to many clinicians who have used high infusion rates of catecholamines in attempts to maintain a falling blood pressure in man. Although it may be possible even during prolonged periods to maintain blood pressure in this way, there is a critical period when the infusion rate is diminished or stopped and the central vasomotor tone is more or less lacking.

Recently Lever, Mowbray, and Peart[59] have studied the postinfusion drop in blood pressure in man and found that after an infusion of norepinephrine for 1 to 2 hours, with a sustained blood pressure of 160 to 190 mm. Hg, the hypotension lasted for up to 2 hours. In 7 hour infusion experiments it was noticed that blood pressure declined during the first 4 hours and then remained steady. This type of infusion was likewise followed by a considerable degree of hypotension. When the skin blood flow was measured with a finger calorimeter during the infusion, a fall was noticed which was further marked during the postinfusion period. The blood flow in the muscles of the forearm was initially decreased with infusion of norepinephrine, whereafter the flow increased to a higher level than the original and remained high even after the end of the infusion. Similar effects have been observed previously with infusion of epinephrine.[95] Lever, Mowbray, and Peart conclude that the effects may be partly explained by the action of a circulating vasodilator, since the increase in flow was not affected by section of the vasomotor nerves. This does not exclude, however, the possibility that norepinephrine like epinephrine, depresses the vasomotor center and the peripheral synapses engaged in the impulse transmission.

Conditions during which epinephrine and norepinephrine have reduced action on the circulatory system. It has been noted by many investigators that during hypoxia blood pressure falls and resumes its previous level, or even a higher one, on adequate admission of oxygen. This effect suggests that the heart and vessels do not respond to the pressor influences during the hypoxic state. Surtshin, Rodbard, and Katz[86] studied this phenomenon and were able to demonstrate that injections of epinephrine had a reduced or no pressor effect during hypoxia in the dog. Similar observations have been made for norepinephrine[22] with control of blood flow. These results may be of considerable significance, as pointed out by the authors, for the treatment of circulatory disturbances with epinephrine and norepinephrine in man.

Like anoxia, hypercarbia greatly lowers not only the activity but also the reactivity of the heart and vessels to chemical stimuli including the neurotransmitter norepinephrine.[73] It would be of interest to study the reactivity of the heart and vessels under hypercarbia at normal pH since Nahas[71] has shown that there is no stimulant action of carbon dioxide on the catecholamine-releasing mechanism if pH is maintained constant at apnea under oxygen, although carbon dioxide tension may attain 80 mm. Hg.

These conditions, during which epinephrine and norepinephrine have been shown experimentally to have a sometimes greatly reduced action, have their counterpart in the clinical experience with epinephrine and norepinephrine treatment in circulatory shock. Depending on the severity of the shock the pressor amines are often more or less ineffective, a phenomenon which has given rise to much discussion and has not always been properly understood. The obvious consequence of this un-

happy lack of action is the necessity of instituting appropriate treatment before the loss of reactivity is irreversibly manifest.

Conditions during which epinephrine and norepinephrine have increased action on the circulatory apparatus. In their pioneer study of the effect of norepinephrine on various circulatory parameters Goldenberg and his co-workers[45] noticed that norepinephrine appeared to exert a stronger action on the blood pressure of hypertensive patients than of normal subjects. A strict comparison of the effects is not possible, since the base line of pressure is different and there is no way to decide whether the effect should be read in percentage increase or absolute increase in pressure. At any rate it was observed that even small doses of norepinephrine raised the pressure more (in millimeters of mercury) in hypertensive than in normotensive subjects. Similar observations have been made by Birke and co-workers[9] and others.

Certain adrenocortical hormones have themselves an ability to increase vascular tone and even produce hypertension. It is therefore not surprising that increased pressor activity of the catecholamines has been noticed after pretreatment with adrenocortical hormones, particularly DOCA,[77] while the effects are reduced after adrenalectomy and in hypocorticalism in man.[25]

An increased pressor response to norepinephrine has been observed in normotensive[38] and in hypertensive patients given hexamethonium.[19,21] The renal vascular bed does not regularly participate in this enhanced pressor response.

The stronger effects of catecholamines after cocaine or hexamethonium have not been fully explained. It may be recalled, however, that any condition causing a depletion of the peripheral norepinephrine stores, such as denervation, or treatment with reserpine, is accompanied by an increased sensitivity or reactivity of the receptor mechanism toward catecholamines. It is possible that the blocking of impulses to postsynaptic adrenergic nerves by hexamethonium has a similar effect.

An interesting difference between hypertensive and normotensive subjects in the response of heart rate to small intravenous doses of norepinephrine has been noted by Judson and associates.[53] They found that while norepinephrine regularly decreases the heart rate in normotensive individuals by inducing a vagus reflex, no change was observed in hypertensive patients. After thoracolumbar sympathectomy, however, the normal response returned. The failing response may be explained by the assumption that in hypertension the homeostatic cardiovascular reflex mechanisms are already largely engaged, resulting in inhibition of the adrenergic nerve impulse outflow. A support for this hypothesis is found in the diminished urinary excretion of catecholamines in hypertensive patients,[9] which, incidentally, seems to rule out norepinephrine or increased adrenergic nerve activity as chief factor in essential hypertension.

Hyperreactivity of the nailfold terminal vascular loops to circulating epinephrine and norepinephrine was observed by Greisman[48,49] in hypertensive patients. Of considerable interest also is the more recent finding of the same author[50] that the reactivity of the nailfold capillary bed to norepinephrine is significantly increased during infusion of angiotonin into subjects with normal cardiovascular systems. The important conclusion is drawn from these observations that angiotonin, unlike norepinephrine, possesses the properties required of the hypothetical humoral pressor substance of essential hypertension as regards effects upon the nailfold capillary bed.

Catecholamine production and release in man measured by plasma levels and urinary excretion

In view of the manifold actions of the catecholamines it is often of interest to obtain a measure of their levels in blood or the total release over a period of time. Attempts to measure the release from the suprarenal gland in man have been made by introducing a catheter into the right ad-

renal vein and to analyze the blood in the samples obtained.[67] A similar technique has been suggested by Ström[32] in order to locate pheochrome cell tumors.

The methods most extensively used for estimation of production and release of epinephrine and norepinephrine have been determination of the concentration level in the blood and the excretion in urine.

Plasma. No attempts will be made in the present article to discriminate between the different fluorimetric methods used for blood or plasma catecholamine estimations, but the reader is referred to the papers by Weil-Malherbe and Bone,[94] Price and Price,[75] Cohen and Goldenberg,[16] García and Wallace,[41] and Vendsalu.[91] An extensive account of the problem is given in a recent monograph by Manger, Wakim, and Bollman.[65]

There are reasons to believe that both methods (trihydroxyindole and ethylenediamine condensation methods) give a relative measure of the catecholamine levels in plasma, as shown by the variations observed in conditions known to be accompanied by typical changes in the epinephrine and norepinephrine content of plasma. The absolute levels are more open to doubt, however, depending on the specificity of the method. This is probably of highest concern as regards the "resting" level.

In using any of the methods for norepinephrine estimations in blood as a measure of catecholamine release, one has to consider some facts which are relevant from a theoretical point of view.

It is generally agreed that the suprarenal gland as well as the adrenergic nerves continuously release epinephrine and norepinephrine in amounts which are very small during rest, particularly in recumbent subjects. The release is proved by the continuous excretion in urine. Any norepinephrine level in blood will of course depend on the amounts released into the blood from the suprarenals and from the adrenergic nerves activated and there is, so far, no reliable means of differentiating between them. On the other hand it is known that the greater part of epinephrine is released from the suprarenals. Of the amounts released, only a part will be found in the peripheral venous or arterial blood, however, since it has to pass through one or two capillary systems involving unknown loss. The norepinephrine level in peripheral venous blood represents the sum of the norepinephrine coming from the arterial blood and the amount released in the vascular region contributory to the vein from which the blood sample was drawn. Obviously this release, and thus the level of norepinephrine in the blood, can vary with the position of the body, the blood flow, and the temperature of the region in question and other local factors which may be of secondary interest only. In measuring the arteriovenous difference in catecholamines it is possible to obtain a minimum figure of the "overflow" from the adrenergic nerves in the region and the influence of various factors upon this. Negative arteriovenous differences were first observed by Weil-Malherbe and Bone[94] and have been verified by several authors.

Again, arterial blood catecholamine levels give essentially the balance between the mean concentration from the different peripheral release areas and the disappearance rate in the pulmonary system.

It should be noted that plasma levels will practically always be so small that pharmacologic actions will be of hardly any significance.

From what has been said it is reasonable to assume that there will be some relationship between the total release and the arterial catecholamine level, but that peripheral venous blood of any single region will be unsuitable for information on the catecholamine production except for that particular region.

Urine. As to the catecholamine excretion in urine, this in all likelihood bears a relationship to the arterial plasma level. By measuring the excretion over a definite period of time an integration of the output will be achieved which gives a relative measure of the production.

The amount excreted in urine is small, only a few per cent of the total amount introduced into the circulation, but seems to be a fairly constant fraction. It has been suggested that other inactivation products such as metanephrine and normetanephrine (the 3-methoxy metabolites of epinephrine and norepinephrine) would be more suitable as basis for an estimation since they appear in urine in larger amounts than the free amines. This is of course based on the assumption that the 3-methoxy-derivatives bear at least as constant a relationship to the total amounts released as the free amines. No data are available on this point, however. One other factor of practical importance is the ease with which the urinary free catecholamines can be quantitatively assayed.[34]

Diagnostic value of urinary catecholamine estimation. The estimation of urinary catecholamine output as a means of diagnosing pheochrome cell tumors was introduced several years ago.[26] It has since been used extensively as a simple and reliable method for this purpose. Epinephrine or norepinephrine figures near and above 100 μg per 24 hours for the free amines are strongly indicative of such a tumor in connection with hypertension, especially in younger individuals.[80] In cases of the paroxysmal type, with normal blood pressure between the attacks, increased figures may be found only in urine collected during and after such periods.

The diagnostic value of increased catecholamine excretion is accentuated by the fact that in essential hypertension the figures are, as a rule, lower than normal.[9]

In postural hypotension the urinary excretion figures are lower than normal, indicating defective functioning of the vasomotor system,[61] a situation which has an analogy in subjects receiving ganglionic blocking agents.[85]

Increased excretion of epinephrine has been observed in mental stress[35] and typically accompanies maniacal behavior.[6]

Only in one other condition have epinephrine and norepinephrine excretion figures been found in the same range as in chromaffin cell tumors, namely, in burns. Goodall, Stone, and Haynes[47] and Birke and associates[10] have reported very high excretion figures and also have pointed out the possible prognostic value of the trend of the excretion figures.

The simple technique of fluorimetric estimation of urinary catecholamines appears at present to be the most suitable method for obtaining information on the catechol release in many clinical conditions and may be complemented by plasma determinations on special occasions, especially in localizing pheochrome cell tumors.

Catecholamines in pathologic conditions

Surprisingly large quantities of catecholamines can be tolerated over a long time, as demonstrated in many cases of pheochrome cell tumors, where the daily secretion from the tumor has been estimated as high as 100 to 200 mg. as against the normal total secretion from the suprarenal gland of less than 1 mg. Since these conditions can last over years it is not likely that either the amines as such or their inactivation products readily cause serious damage to the organism. Such effects might be expected to occur first in the cardiovascular system. This is possibly of interest since it has been claimed that accumulation of catecholamines in the heart may contribute to cardiac damage and atherogenesis.[76] There is evidence in animal experiments that administration of large doses of catecholamines, particularly epinephrine, can cause such damage, but it is still uncertain whether increased release has similar effects in man.

In a recent symposium in Burlington, Vermont, some problems related to the catecholamines in cardiovascular disease have been extensively considered and their potentially pathogenic properties and prominent role in the origin of functional and degenerative cardiovascular diseases emphasized.

A survey of local and systemic pathologic changes after epinephrine and norepineph-

rine administration has recently appeared.[89]

The statement that 2 hours after the administration of adrenaline large amounts of the amine were found in the heart and spleen, to indicate that these tissues not only accumulate the hormone, but also retain it for long periods of time,[2] apparently refers to very large doses. It was found in the cat[28] that a dose as high as 5,000 μg did not cause any increase in the catecholamine content of the tissue. Bing and Kako[8] found that addition of very small amounts of norepinephrine or dopamine to the fluid perfusing the heart-lung preparation increased the efficiency of the heart, while large quantities were conducive to heart failure. Dury and Werthessen[23] found a decreased biosynthesis of cholesterol in the aorta following epinephrine. Maling and Highman[63] concluded from their experiments that it was unlikely that the norepinephrine in the heart had a significant role in the production of spontaneous ventricular arrythmias and the prolonged ventricular hypersensitivity after coronary ligation.

From the data available at present it emerges that very large quantities of epinephrine and possibly norepinephrine may be deleterious to cardiovascular function. These amounts will, however, hardly be of psychological relevance, and should, if possible, be avoided when catecholamines are used for therapeutic purposes.

References

1. Alexander, J. K., Mise, J., Dennis, E. W., and Hershberger, R. L.: Circulation, **18**:235-248, 1958.
2. Axelrod, J., and Weil-Malherbe, H.: Abstr. Int. Symposium on Catecholamines, Vermont, 1959.
3. Barclay, J. A., Cooke, W. T., and Kenney, R. A.: Am. J. Physiol. **151**:621-625, 1947.
4. Barcroft, H., and Konzett, H.: J. Physiol. **110**:194-204, 1949.
5. Bearn, A. G., Billing, B., and Sherlock, S.: J. Physiol. **115**:430-441, 1951.
6. Bergsman, A.: Acta psychiat. et neurol. scandinav. **34**:Suppl. 133, 1959.
7. Biberfeld, J.: Med. Klin. pp. 1177-1179, 1906.
8. Bing, R. J., and Kako, K.: Abstr. Int. Symposium on Catecholamines, Vermont, 1959.
9. Birke, G., Dunér, H., Euler, U. S. von, and Plantin, L. O.: Ztschr. Vitamin-Hormon-Ferm. Fortsch. **9**:41-68, 1957.
10. Birke, G., Dunér, H., Liljedahl, S. O., Pernow, B., Plantin, L. O., and Troell, L.: Acta chir. scandinav. **114**:87-98, 1957.
11. Bonvallet, M., Dell, P., and Hiebel, G.: Electroencephalog. & Clin. Neurophysiol. **6**:119-144, 1954.
12. Bovet, D., Virno, M., Gatti, G. L., and Carpi, A.: Arch. int. pharmacodyn. **110**:380-409, 1957.
13. Brodie, B. B., Spector, S., and Shore, P. A.: Pharmacol. Rev. **11**:548-564, 1959.
14. Bülbring, E., and Burn, J. H.: J. Physiol. **101**:289-303, 1942.
15. Catchpole, B. N., Hachel, D. B., and Simeone, F. A.: Ann. Surg. **142**:372-381, 1955.
16. Cohen, G., and Goldenberg, M.: J. Neurochem. **2**:58-70, 1957.
17. Cohen, G., and Goldenberg, M.: J. Neurochem. **2**:71-80, 1957.
18. Cohen, S. I., Silverman, A. J., and Zuidema, G.: A.M.A. Arch. Neurol. & Psychiat. **76**:670-674, 1956.
19. Corcoran, A. C., Wagner, W. E., and Page, I. H.: J. Clin. Invest. **35**:868-873, 1956.
20. Crawford, E. S., and Haynes, B. W., Jr.: Am. J. Surg. **19**:191-201, 1953.
21. Doyle, A. E., and Black, H.: Circulation, **12**:974-980, 1955.
22. Dunér, H., and Euler, U. S. von: Acta physiol. scandinav. **46**:201-208, 1959.
23. Dury, A., and Werthessen, N. T.: Abstr. Int. Symposium on Catecholamines, Vermont, 1959.
24. Elmadjian, F., Hope, J. M., and Lamson, E. T.: Recent Progress in Hormone Research, **14**:513-553, 1958.
25. am Ende, S.: Ztschr. klin. Med. **136**:108-115, 1939.
26. Engel, A., and Euler, U. S. von: Lancet, **2**:387, 1950.
27. Euler, U. S. von: Noradrenaline, Springfield, Ill., 1956, Charles C Thomas, Publisher.
28. Euler, U. S. von: Circulation Res. **4**:647-652, 1956.
29. Euler, U. S. von: Third Int. Congr. Cardiol., Brussels, 1958.
30. Euler, U. S. von: Am. Heart J. **56**:469-477, 1958.
31. Euler, U. S. von, Franksson, C., and Hellström, J.: Acta physiol. scandinav. **31**:1-5, 1954.
32. Euler, U. S. von, Gemzell, C. A., Ström, G., and Westman, A.: Acta med. scandinav. **153**:127-136, 1955.
33. Euler, U. S. von, and Lishajko, F.: Acta physiol. et pharmacol. neerl. **6**:295-303, 1957.
34. Euler, U. S. von, and Lishajko, F.: Acta physiol. scandinav. **45**:122-132, 1959.

35. Euler, U. S. von, and Lundberg, U.: J. Appl. Physiol. **6**:551-555, 1954.
36. Euler, U. S. von, and Purkhold, A.: Acta physiol. scandinav. **24**:212-217, 1951.
37. Fowler, N. O., Westcott, R. N., Scott, R. C., and McGuire, J.: J. Clin. Invest. **30**:517, 1951.
38. Freis, E. D., MacKay, J. C., and Oliver, W. F.: Circulation, **3**:254-259, 1951.
39. Funkenstein, D. H., Greenblatt, M., and Solomon, H. C.: Psychosom. Med. **14**:347-362, 1952.
40. Gaddum, J. H., and Holzbauer, M.: Vitamins & Hormones **15**:151-203, 1957.
41. Garcia, U. H., and Wallace, J. M.: Abstr. Twenty-first Int. Congr. of Physiol. Sciences, Buenos Aires, 1959.
42. Gazes, P. C., Goldberg, L. I., and Darby, T. D.: Circulation **8**:883-892, 1953.
43. Gilmore, J. P.: Am. J. Physiol. **195**:473-475, 1958.
44. Glover, W. E., Greenfield, A. D. M., Kidd, B. S. L., and Whelan, R. F.: J. Physiol. **140**:113-121, 1958.
45. Goldenberg, M., Pines, K. L., Baldwin, E. F., Greene, D. G., and Roh, C. E.: Am. J. Med. **5**:792-806, 1948.
46. Goodall, McC.: Acta physiol. scandinav. **24**: suppl. 85, 1951.
47. Goodall, McC., Stone, C., and Haynes, B. W., Jr.: Ann. Surg. **145**:479-487, 1957.
48. Greisman, S. E.: J. Clin. Invest. **31**:782-788, 1952.
49. Greisman, S. E.: J. Clin. Invest. **33**:975-983, 1954.
50. Greisman, S. E.: J. Exper. Med. **103**:477-486, 1956.
51. Helve, O., and Pekkarinen, A.: Ann. med. exper. fenniae **30**:337-360, 1952.
52. Holzbauer, M., and Vogt, M.: J. Neurochem. **1**:8-11, 1956.
53. Judson, W. E., Epstein, F. H., and Wilkins, R. W.: J. Clin. Invest. **29**:1414-1420, 1950.
54. Kety, S. S.: Science **129**:1528-1532, 1959.
55. King, B. D., Sokoloff, L., and Wechsler, R. L.: J. Clin. Invest. **31**:273-279, 1952.
56. King, S. E., and Baldwin, D. S.: Am. J. Med. **20**:217-224, 1956.
57. Lansing, A. M., and Stevenson, J. A. F.: Am. J. Physiol. **193**:289-293, 1958.
58. Lee, W. C., and Shideman, F. E.: Science **129**:967-968, 1959.
59. Lever, A. F., Mowbray, J. F., and Peart, W. S.: J. Physiol. **146**:43P-44P, 1959.
60. Livesay, W. R., and Chapman, D. W.: Am. J. M. Sc. **225**:159-171, 1953.
61. Luft, R., and Euler, U. S. von: J. Clin. Invest. **32**:1065-1069, 1953.
62. Luisada, A. A., Liu, C. K., Jona, E., and Polli, J. F.: Angiology **6**:503-504, 1955.
63. Maling, H. M., and Highman, B.: Abstr. Int. Symposium on Catecholamines, Vermont, 1959.
64. Malméjac, J., Chardon, G., Neverre, G., and Gross, A.: Compte rend. Soc. biol. **148**:536-539, 1954.
65. Manger, W. M., Wakim, K. G., and Bollman, J. L.: Chemical Quantitation of Epinephrine and Norepinephrine in Plasma, Springfield, Ill., 1959, Charles C Thomas, Publisher.
66. Marrazzi, A. S.: Exper. Cell Res. Suppl. 5, pp. 370-385, 1958.
67. Masoni, A.: Acta med. scandinav. **159**:225-234, 1957.
68. Miller, A. J., Shifrin, A., Kaplan, B. M., Gold, H., Billings, A., and Katz, L. N.: J.A.M.A. **152**:1198-1201, 1953.
69. Moyer, J. H., Morris, G., and Snyder, H.: Circulation **10**:265-270, 1954.
70. Moyer, J. H., Skelton, J. M., and Mills, L. C.: Am. J. Med. **15**:330-343, 1953.
71. Nahas, G. G.: Compte rend. Acad. sc. **248**:294-297, 1959.
72. Paasonen, M. K., and Krayer, O.: Experientia **15**:75, 1959.
73. Page, I. H., and Olmsted, F.: Circulation, **3**:801-819, 1951.
74. Patel, D. J., Lange, R. L., and Hecht, H. H.: Circulation **18**:19-24, 1958.
75. Price, H. L., and Price, M. L.: J. Lab. & Clin. Med. **50**:769-777, 1957.
76. Raab, W., and Gigee, W.: Circulation Res. **3**:553-558, 1955.
77. Raab, W., Humphreys, R. J., and Lepeschkin, E.: J. Clin. Invest. **29**:1397-1404, 1950.
78. Reis, G. von: Opusc. med. Stockholm **4**:50-52,
78a. Roberts, J., Olichney, M. J., Roughgarden, J. W., and Modell, W.: Miami Meeting of Pharmacology, 1959.
79. Rushmer, R. F., and West, T. C.: Circulation Res. **5**:240-246, 1957.
80. Sack, H., and Koll, J. F.: Die Erkennung, Beurteilung und Behandlung des symptomatischen Hochdrucks, Stuttgart, 1959, F. Enke, Verlag.
81. Salzman, E. W., and Leverett, S. D., Jr.: Circulation Res. **4**:540-545, 1956.
82. Sampson, J. J., and Zipser, A.: Circulation **9**:38-47, 1954.
83. Scherf, D.: Abstr. Int. Symposium on Catecholamines, Vermont, 1959.
84. Sensenbach, W., Madison, L., and Ochs, L.: J. Clin. Invest. **32**:226-232, 1953.
85. Sundin, T.: Acta med. scandinav. **161**:Suppl. 336, 1958.
86. Surtshin, A., Rodbard, S., and Katz, L. N.: Am. J. Physiol. **152**:623-632, 1948.
87. Swan, H. J. C.: J. Physiol. **112**:426-437, 1951.
88. Swan, H. J. C.: Brit. M. J. **1**:1003-1006, 1952.

89. Szakacs, J. E., Dimmette, R. M., and Cowart, E. C., Jr.: Research Report, U. S. Naval Med. School, Bethesda, Md., 1959.
90. Thorn, G. W., Jenkins, D., and Laidlaw, J. C.: Recent Progress in Hormone Research, **8**:171-215, 1953.
91. Vendsalu, A.: Kungl. Fysiograf. Sällsk. Lund Förhandl. **29**:45-50, 1959.
92. Walton, R. P., Walton, R. P., Jr., and Thompson, W. L.: J. Pharmacol. & Exper. Therap. **125**:202-207, 1959.
93. Waud, D. R., and Krayer, O.: Abstr. Int. Symposium on Catecholamines, Vermont, 1959.
94. Weil-Malherbe, H., and Bone, A. D.: Biochem. J. **58**:132-141, 1954.
95. Whelan, R. F.: J. Physiol. **118**:575-587, 1952.
96. Wiggers, C. J.: Circulation Res. **2**:271-279, 1954.
97. Wolff, F. W.: Lancet, **2**:72-73, 1954.

This feeling, that we are always on the verge of some great clarification is, I believe, a powerful element in the fascination which this problem of the relation between action and constitution has exercised on many minds. We have plenty of stimulating suggestions and seductive analogies—half-lights and elusive hints. Progress towards real clarity of conception can only come from the continuous cooperation and intercourse between the workers in all the different departments of scientific inquiry with which the problem makes contact. Particularly, I think, is it necessary to realize that the core of the problem is physiological . . . . We need all the help that organic and physical chemistry can give, but the initiative and the guiding influence in the inquiry must come at least as much from the physiological side. Under such conditions I believe the quest is well worthy of an eager pursuit. It is not merely a question of finding a scientific basis for the production of more and better therapeutic agents than those which are yet available. There is a whole group of physiological and pathological problems here closely knit together, and, from whatever quarter the light comes, it cannot fail to illuminate the whole field.

FROM "CHEMICAL STRUCTURE AND PHYSIOLOGICAL ACTION," BY H. H. DALE
BULLETIN OF JOHNS HOPKINS HOSPITAL, **31**:373-380, OCTOBER, 1920.

Newer drugs in amebiasis

Amebiasis, a systemic disease due to Endamoeba histolytica, is not confined to the tropics. Its incidence reflects the sanitary level of the environment.

In the colon, associated bacteria are believed to play a role in its pathogenesis, but not in the liver and other extraintestinal tissues. Realistically, the therapeutic regime depends upon the site of amebic invasion, and whether or not associated organisms are believed to be contributory to the disease complex. "Specific" amebicides are available, capable of clearing the majority of cases, i.e., of chronic amebiasis as seen in the United States. In a minority, however, recurrence of infection, due to amebae with or without associated bacteria, is persistent. To combat recurrence, especially in environments of high endemicity, personal and community hygiene is mandatory.

Newer drug types currently under trial are: glycobiarsol, N.F.; representative dichloracetamides, of which Mebinol is an example; certain phenanthrolines, such as Entobex; diamino derivatives, such as MA-307; and paromomycin (Humatin), an antibiotic. Suppressive therapy and adequate hygienic precautions provide a more enlightened approach to the control of amebiasis. No one therapeutic agent, or combination, is indicated universally in combating this disease.

Hamilton H. Anderson, M.D. *San Francisco, Calif.*
Department of Pharmacology and Experimental Therapeutics, School of Medicine, University of California, San Francisco

Amebiasis, a systemic disease due to *Endamoeba histolytica*, is by no means confined to the tropics. It is prevalent in the environment, regardless of latitude, where personal, community, or institutional hygiene is not adequately controlled. Thus, one sees in the United States a high incidence of chronic or "carrier" amebiasis in institutions for the mentally ill, or in communities where adequate sanitation is not maintained. Like typhoid fever in past decades, amebiasis now reflects directly on the sanitation level of the area.

Oftentimes remote areas, particularly in the tropics, may have high levels of infection but, with improved sanitation, as in the canal zone of Panama, the incidence does not differ appreciably from that noted in southern parts of the United States. On the other hand, amebiasis was first described in St. Petersburg in 1875 by Lösch, and currently there is reported to be a high incidence in Siberia.[1]

Since amebiasis constitutes a pathologic state with diffuse invasion of the mucosa of

Supported, in part, by the National Institutes of Health, Bethesda, Md.

the large bowel, accompanied by tissue lysis, there is little evidence of an inflammatory reaction. The tissue most frequently invaded beyond the bowel is the liver, where abscess formation may occur without bacterial involvement. But in the lumen of the bowel, on the other hand, the bacterial flora may influence the invasive propensity of the ameba. Again, in the tissue phase of amebiasis only the trophozoites are found, whereas in the lumen of the colon either cysts (in chronic or "carrier" states) or trophozoites in acute dysentery may occur. Thus, one finds the therapeutic problem rather complex, depending on the site of invasion, presence or absence of associated organisms, as well as cystic and trophic forms to be combated. One does not wonder that there is no single agent available which is completely effective in the hands of experienced investigators working in various clinics of the world.

To emphasize this point from the therapeutic aspect, we may cite evidence of the action, oftentimes dramatic, of emetine in hepatic amebiasis, including abscess formation. Contrariwise, the bowel harboring *E. histolytica* is cleared in only about one-third of cases. Chloroquine has a similar action on amebae in the liver, and not in the bowel. On the other hand, we have agents like fumagillin and the tetracyclines, notably Terramycin, which may clear the bowel of ameba but not act on the same organisms residing in the liver. Anderson[2] and others have reported the development of hepatic amebiasis during the course of therapy with either of these two antibiotics.

Further, to evaluate the role of associated bacteria and their participation in the etiology of intestinal amebiasis, Phillips and Wolfe[3] have obtained results which provide additional evidence. They believe that "some unidentified factor or factors contributed by bacteria seemed to be essential for the maintenance of *E. histolytica* in the intestine and, possibly, to the pathogenesis of amoebic disease." Adams[4] came to a similar conclusion in 1943, based on his clinical experience with intestinal disease and its response to therapy. Thus, we are faced with the impression that amebae with their associated bacteria in the colon present an entirely different therapeutic problem from that encountered when amebae, without associated bacteria, invade hepatic tissue. Realistically, we should develop any therapeutic regime on this premise, clearing the initial focus in the bowel, and then combating amebae in the liver or other invaded tissues, after evidence of such invasion is manifest. Blanket or "combined therapy" for liver involvement is difficult to justify in the United States.

Thus, it is difficult to define an ideal amebicide, which will combat amebae under different conditions of growth, in people residing in various parts of the world who respond differently to *E. histolytica.* Certainly, peroral administration is desirable, effectiveness against trophozoites and cysts in the bowel required, as well as adequate absorption to combat systemic disease such as liver involvement when it occurs. While such a regime with an "ideal" agent will undoubtedly clear the acutely ill or chronically infected patient of *E. histolytica,* there is the problem of suppression of disease in those continually or intermittently exposed to "cyst passers." For such suppressive therapy, one should have an agent which is cyst destroying, and yet not absorbed to a degree which would endanger the host should daily use over months or years be required.

Finally, before we discuss specific agents against amebiasis, one should define diagnostic criteria generally agreed upon as necessary. Too often, in patients who have once had a diagnosis of harboring *E. histolytica,* the treatment is carried far beyond reasonable limits, and is repeated by every subsequent physician who may believe the symptom complex relates to the ameba. First, one should be certain that the patient has *E. histolytica,* which can best be demonstrated in iron-hematoxylin-stained fecal specimens. A series of six, taken at daily intervals, if not positive, should be followed

by proctoscopy and scrapings of the bowel wall which may reveal the amebae.

Sigmoidoscopic examination should disclose some evidence of ulceration in approximately half of the patients infected. A barium enema and radiologic examination often reveal defects higher in the bowel, notably in the cecal area. Symptoms alone are not adequate reasons for antiamebic therapy.

Once the diagnosis is established and the appropriate therapy used, then adequate follow-up is mandatory. Within a few weeks of completion of therapy, sigmoidoscopic examination should be repeated. Specimens of feces, from three to six at daily intervals, if found to be negative, together with a cleared mucous membrane of the large bowel, are considered a satisfactory response. At 3 to 6 month intervals the examination of the bowel and of the series of stools should be repeated. Arbitrarily, at 6 months, with no recurrence of symptoms or signs of the disease, no further therapy is warranted. For the diagnosis of hepatic involvement, the procedure is based largely on clinical acumen and familiarity with the disease. One should consult the reports of Wilmot and his co-workers[5] in 1951 and later for procedures employed. The demonstration of *E. histolytica* need not be required in extraintestinal disease.

Choice of current therapy

While there are still some who favor combined therapy with the inclusion of potentially toxic agents, such as use of emetine in chronic amebiasis,[6] it is believed desirable to avoid mixed, "shotgun" regimes over protracted periods. Especially is this true in the majority of patients with amebiasis in this country, i. e., the chronic "cyst-passer," with or without symptoms or signs of disease. It is very easy to overtreat these individuals, in the belief that a variety of drugs given at one time insures against recurrence of the disease. There is no series of comparative studies in man which support this point of view. Rather, one should select the agent most likely to be effective, and then proceed in steplike fashion to other chemical types, on the basis of demonstration of *E. histolytica* in addition to relevant signs and symptoms of disease, until eradication is accomplished.

There is a plethora of amebicides currently in use, of which the following are perhaps the best examples. Other therapeutic equivalents could be included, but offer little or no advantage over these against *intestinal amebiasis:*

1. Carbarsone, U.S.P. (or Thiocarbarsone*)
2. Iodochlorhydroxyquin (Vioform), N.F.
3. Fumagillin, N.N.D.
4. Erythromycin, U.S.P.
5. Tetracyclines or bacitracin, U.S.P.

Against hepatic amebiasis, chloroquine phosphate, U.S.P., has largely replaced emetine. Only when recurrence of infection takes place (25 per cent of cases[5]) is one justified in using emetine in nonambulatory patients.

Each of these orally acceptable agents is capable of acting systemically, except fumagillin and bacitracin, which are absorbed in minimal amounts. On absorption one should be concerned about arsenic and its effect in concurrent liver and kidney disease, when carbarsone is used. Likewise, in the use of Vioform, the iodine content (41.54 per cent) and chlorine (11.61 per cent) may contribute to hepatic damage when the drug is used in excessive amounts. It is noteworthy, however, that outside the United States this agent is used freely by laymen oftentimes without supervision, and yet is seldom, if ever, incriminated in reports of toxicity.

Fumagillin is unique among the amebicides in current use in that its activity in vitro and in laboratory infected animals exceeds that of all others, including emetine. Further, among the antibiotics, it has no demonstrable antibacterial activity. Despite these qualifications it has not proved to be

*This is a more toxic thioarsenite (trivalent) analog of carbarsone, which is available commercially.[7]

more effective than other antibacterial antibiotics, such as the tetracyclines, which in turn have little direct action against the ameba that can be demonstrated in the laboratory. In addition, when either is used in excessive amounts, untoward effects may occur. Therefore, fumagillin should be considered when other agents have failed to clear the intestine of amebae, and it is indicated in those cases where associated bacterial flora does not play an important role.

Of the antibacterial antibiotics of demonstrated effectiveness in intestinal amebiasis, erythromycin may be preferred. Two reasons for this are its inherent safety and absorption and its apparent value in combating or preventing hepatic disease.[8] An equally important aspect is its usefulness in patients who have extensive ulceration of the large bowel, due presumably to the part played by associated bacteria. Therefore, one may turn to the use of erythromycin (or one of its numerous relatives under their protected names), when bacteria are thought to provide a significant feature of the patient's disease complex.

The tetracyclines, notably Aureomycin and Terramycin, have been extensively exploited as "specifics" in amebiasis. They do little more than alter the bacterial flora, however, providing an adversely favorable medium within the lumen of the bowel for the growth of *E. histolytica*. In patients followed over a period of months, it has been noted that gradual return of the flora to the pretreatment level parallels return of the ameba, and relapses are frequent following such nonspecific therapy.[9]

Bacitracin, with its penicillin-like spectrum of activity in the bowel, has been proposed, in combination with a direct-acting amebicide (carbarsone), and used with some success. The reasons for its activity, which is nonspecific and essentially antibacterial, are similar to those cited for the tetracyclines.

Thus, in re-evaluation of the use of accepted amebicides one should decide on the basis of need as exhibited by the patient the following: (1) Is the disease complex caused by amebae in the colon confined to this area, and accompanied by ulceration and evidence of tissue invasion? If so, then a locally acting absorbable amebicide is indicated. (2) Is the disease complicated by associated microorganisms, in part responsible for the signs and symptoms? If so, then not only a direct-acting amebicide, but an essentially antibacterial agent is indicated as well. (3) Finally, is the ameba causing tissue damage beyond the bowel? If so, then one turns to other agents.

As a footnote of caution we must recognize the potential toxicity of carbarsone, one of the safest arsenicals now employed in therapy of amebiasis. Radke and Baroody[10] have reviewed the literature, and report 45 cases up to 1957 encountered in 1,500 patients treated by them. Fever, epigastric burning, abdominal pain, diarrhea, nausea and vomiting were most frequently noted. Withdrawal of the drug and, in some, use of BAL (dimercaptopropanol) were effective. Exfoliative dermatitis developed in 3, in 5 there was central nervous system involvement, and in one liver damage was noted as well. In another unreported series, where carbarsone was used in mass prophylaxis without adequate medical supervision, encephalopathy was encountered, and unexplained deaths noted.* While carbarsone is still regarded as a safe drug under medical supervision it should not be used promiscuously.

Newer drugs under study

Among the many drugs of natural origin and newer chemical derivatives currently under study,† the following have appeared to warrant some continued use in experimental therapy:

Glycobiarsol (Milibis), N.F. This agent contains 15 per cent arsenic and 42 per cent bismuth.

Mantomide [N-(2,4-dichlorobenzyl, N-2-hydroxyethyl)-dichloroacetamide]; Enta-

*Nelson, T. L.: Personal communication.

†Elslager, E. F.: Review on Anti-amebic Agents. In Burger, Alfred: Medicinal Chemistry, New York, Interscience Publishers, Inc. In press.

mide [dichloroacet-4-hydroxy-N-methylanilide]; and Mebinol [N-(β-oxyethyl)] N-[p-phenoxy-(4 nitro benzyl) dichloroacetamide].

Entobex (11,925-C-Ciba) [5,6-quinone, 4,7-phenanthroline].

MA-307 [N,N′-dipiperonyl-5,11-diaminopentadecane-2 HCl].

Paromomycin (Humatin), an antibiotic.

Glycobiarsol, N.F. This agent has had extensive trials during the past 5 years, usually in combination with other agents. Since it contains arsenic, there is some concern regarding possible toxicity. However, less than 2 per cent arsenic can be accounted for in distribution and excretion studies. Nevertheless, encephalopathy has been observed.[11]* Because of these possibilities, chronic therapy with this or other "suppressive" agents against cyst-passers in amebiasis is restricted to periods of one month or less. The low solubility and poor absorption (according to N.N.D.) limit the usefulness of glycobiarsol to the prevalent intestinal form of the disease.

In more recent controlled studies in Chile, Silva and associates,[12] after treating 93 patients with chronic intestinal amebiasis with tetracycline or chloroquine plus bismuth glycolylarsanilate (glycobiarsol), found that only 38 individuals remained negative for *E. histolytica.* They conclude, rightly, that reinfection plays an important role in this disease; so failure cannot be attributed solely to drugs. In this report, an epidemiologic survey showed gross carelessness in personal hygiene and poor home sanitation, particularly in those patients who exhibited recurrent infections.

Mass therapy in the United States, with attempts to control amebiasis in mental and other institutions, has met with indifferent success. Despite earlier reports to the contrary,[13] Brooke and associates[9] in 1958 found "the effectiveness of therapy appeared to be related to the prevalence rate of *E. histolytica* . . . and to the general level of personal hygiene of the patients." No significant differences were found between carbarsone, iodochlorhydroxyquin, oxytetracycline, and a combination of oxytetracycline and carbarsone. The incidence of infection rose to one-half of the pretreatment level by the end of 6 months. They cite the experience of Berberian and co-workers[14] who reported, after mass therapy with bismuth glycolylarsanilate (glycobiarsol) and chloroquine phosphate administered weekly, a reduction in infection rate of *E. histolytica* (in another institution) to 25 per cent. Daily administration to another group reduced the rate to 10 per cent of pretreatment levels.

Current studies, as yet unreported,* in an institution for mental defectives, suggest immediate reduction of the prevalence rate to from 60 to 80 per cent of the former level, with the use of glycobiarsol and chloroquine. Improvement in sanitary facilities, including instruction to attending personnel in sanitary techniques, and controls were carried forward simultaneously. All food handlers were treated prophylactically. In some wards, where patients proved to be refractory to therapy, other agents such as iodochlorhydroxyquin (Vioform) were required. It is believed that the drugs used *in addition to controlled sanitation* have reduced the incidence of the disease to lower levels than those encountered in patients being admitted from outside the institution.

Not only institutionalized patients in this country, but people traveling overseas in highly endemic areas are in need of appropriate prophylactic (or suppressive) therapy. In 1958 the recommendation was reemphasized[15] that glycobiarsol (Milibis) should not be used for longer than one month. Other agents, notably iodochlorhydroxyquin, have been reported by McPhee[16] to be useful in 361 subjects attached to the United States team during Pan-American games in Mexico City in 1955. Among this treated group there were 49 men who developed enteritis, 3 cases of which were sufficiently severe to warrant bed rest. Fif-

*Nelson, T. L.: Personal communication.

*Nelson, T. L.: Personal communication.

teen women developed diarrhea. No bloody stools were noted in either of these groups. It was believed that the incidence of enteric infections was much less in those taking Vioform.

A contrary opinion on "Diarrhea of Travelers," without relating such diarrhea to the presence of *E. histolytica*, has been expressed by Kean and Waters.[17] Again in Mexico City, North American students were the subjects treated. No effort was made to start prophylaxis before or immediately upon arrival. Yet the authors conclude that, after a period of 2 weeks' therapy, diarrhea occurred in 38.6 per cent of those who took iodochlorhydroxyquin, in 20.1 per cent of those who took neomycin, and in 33.6 per cent of those who took a placebo. The relevance of this study to suppression of amebiasis was not established.

Dichloro-derivatives. Because of uncertainty as to the efficacy of currently available agents, particularly for use in chronic (cyst-passer) amebiasis and in suppression of the disease in highly endemic environments, newer chemical types are currently under study. Among these, certain dichloroderivatives are of more widespread interest, especially outside the United States.

Mantomide. According to Elslager* the first compound of this type selected for extensive study was Win 5047 (Mantomide). In vitro and in some artificially infected animals it proved to be effective as an amebicide. It was well tolerated in all species. Although absorbed, much of the drug undergoes metabolic alteration. In man, however, it eliminates cysts of *E. histolytica* in asymptomatic individuals. It is distinctly less effective in symptomatic amebiasis and in hepatic amebiasis it has been reported ineffective. Most recently (1959), Donckaster and his collaborators[18] have reported on the use of this agent in 80 cases of chronic amebiasis. Three parasitologic control examinations were performed every other day 10, 30, and 90 days after therapy. Approximately 50 per cent of the patients had negative examinations on the tenth and ninetieth days. After utilizing various therapeutic schemes, the authors conclude that the use of Pontalin (Mantomide) in the treatment of chronic amebiasis is not justified.

Entamide. Entamide (R.D. 3803), a trade-named preparation of Boots Pure Drug Company of England, is dichloroacet-4-hydroxy-N-methylanilide. On clinical trial,[19] Entamide likewise was more effective in asymptomatic patients harboring *E. histolytica* than against trophozoites in patients who exhibited symptoms of disease. The drug is reported to be more active against infections in which cysts of the large race are present, according to Woodruff, Bell, and Schofield.[19]

Mebinol. Mebinol [N-(β-oxyethyl)]N-[p-phenoxy-(4-nitro benzyl) dichloroacetamide] is the most recent of the dichloroacetamide derivatives to be exploited clinically. de Carneri[20] and his collaborators in 1958 and later have expressed the belief, based on in vitro studies and on therapy of artificially infected rodents, that this derivative may be approximately four times as effective as Mantomide in man. When it is used in 0.5 Gm. amounts orally thrice daily over 10 or more days, cyst-passers are rid of *E. histolytica*.[17] The same doses given twice weekly are under study by de Carneri and others as a suppressive.* Results elsewhere, notably in India, reported by Mehta and coworkers[21] suggest, again, that this agent is less active in acute and subacute amebiasis. After daily therapy as noted, a one-week follow-up revealed a cure rate of 77 per cent. No toxic effects were reported. It appears at present that Mebinol may become the more active of the dichloroacetamides, especially in suppressive therapy.

Entobex. This drug (11,925-C or 5,6-quinone-4,7-phenanthroline) and its semicarbazone relative (11,925-Ciba) have been under study in India and elsewhere since 1955.[22] The semicarbazone as well as the

*Elslager, E. F.: Review on Anti-amebic Agents. In Burger, Alfred: Medicinal Chemistry, New York, Interscience Publishers, Inc. In press.

*de Carneri, Ivo: Personal communication.

parent compound were both active in vitro and in laboratory infected rats in tolerated amounts (50 mg. per kilogram). In man, however, 11,925 is less well tolerated than Entobex, although the former agent is more active in hepatic amebiasis.[23,24] Both agents provided control of diarrhea in Indian patients, and each was shown to be bacteriostatic against representative gram-negative and gram-positive organisms. Chowdhury and associates[24] reported that in oral dose range up to 600 mg. per day, compound 11,925 caused nausea and vomiting in 18 of 21 cases between the first and fourth days of therapy. Entobex, on the other hand, was better tolerated, but highly colored urine appeared in 4 of 40 cases.*

In the 21 patients given drug 11,925, clinical "cure" was reported in 12 and symptomatic relief in 3. In 40 patients given 11,925-C (Entobex), clinical "cure" was achieved in 30 and symptomatic relief in 2. Treatment continued daily over from 7 to 10 days. In 9 cases both drugs were combined, with clinical "cure" in 5 and symptomatic relief in 2. In the total series were 18 patients with hepatic manifestations which responded favorably, according to Indian reports. It would appear that Entobex (11,925-C) is less active against cysts of *E. histolytica* than against trophozoites.

Clinical trials have continued in Egypt[25] with follow-up studies up to 21 days. The impression of Nagaty and his co-workers is that this drug seems ineffective in amebic hepatitis. A longer period of follow-up would be required to be certain of the effectiveness of Entobex in intestinal amebiasis. Other studies, reported by Belgian authors† and in Italy[26] and in Brazil,[27] may clarify the ulti ate place of these representative phenanthroline compounds in the treatment of amebiasis.

MA-307. A totally different chemical class, the diamines, have been studied by Beppler and Schweiger[28] and, among these, MA-307 (N,N′-dipiperonyl-5,11-diaminopentadecane-2 HCl) proved to be active in vitro within the range of emetine. This was confirmed in in vitro and in vivo studies of naturally infected macaques and in man. While weight loss occurred in 3 of 6 macaques during therapy, at oral doses of from 25 to 75 mg. per kilogram, no other untoward effects were noted except diarrhea during the first 5 days of the 10 day course. No impairment of function was noted in clinical-laboratory studies.[29]

Initially, in man, oral doses of from 1 to 6 mg. per kilogram daily were given over 5 days to 33 patients who harbored the cysts of *E. histolytica.* Nineteen of these patients exhibited enlarged livers. During the first month's follow-up 31 of 33 patients were cleared of amebae; during the second, 25 of 33; and at the end of follow-up (3 months) 20 of 33 were negative for *E. histolytica.* The liver was reduced in size in two-thirds of the cases, and weight gains were noted in approximately the same number.

In an extension of this study to more fulminating disease, MA-307 was used in Bantus suffering from acute intestinal amebiasis. The oral dose was 600 mg. daily over 10 days, and in some patients (7 of 11) emetine in two-thirds the usual dose was used as well. Utilizing the criteria of Wilmot, Armstrong, and Elsdon-Dew,[5] the authors noted clearance of *E. histolytica* and healing of sigmoid ulcers in 9 of 11 patients followed during 27 days in the hospital. A comparison between emetine and MA-307 did not show that the combination resulted in more satisfactory response than the use of emetine alone in higher dose levels.[30] No serious side effects were noted. In other series of 10 patients given 100 mg. thrice daily for 10 days, under less well-controlled conditions in the hospital, healed ulcers resulted and all but 2 patients were cleared of *E. histolytica* when discharged from the hospital.

While no conclusions are warranted from published data on MA-307, it would appear

---

*Chatterjee[23] reports that in large oral doses in dogs vomiting and hematuria occurred.

†Elslager, E. F.: Review on Anti-amebic Agents. In Burger, Alfred: Medicinal Chemistry, New York, Interscience Publishers, Inc. In press.

possible that in cases which require emetine hydrochloride beyond the amounts usually recommended, MA-307 might be considered as a less toxic agent. Its use above, however, in acute fulminating African amebiasis was not adequate, in the opinion of Wilmot.*

Paromomycin (Humatin). Among the newer antibiotics paromomycin (Humatin), which exhibits broad antibacterial activity, has been reported in the treatment of amebic dysentery by Shafei.[31] It is said to have controlled experimental infections in rats and dogs and to have given better results than similar doses (6 to 25 mg. per kilogram daily in dogs) of neomycin. No damage to kidneys, liver, or bone marrow was reported following oral use over 7 weeks in dogs. Absorption from the gut is limited. Of Shafei's 20 human patients in Egypt, 8 had acute symptoms and 12 gave histories of recurrent episodes of dysentery. All complained of diarrhea, blood and mucus, and their stools were reported to be positive for amebae and/or cysts. Sigmoidoscopy at the outset and during 3 months following treatment was more helpful in follow-up "than the clinical picture or stool examination." All received either 10 mg. per kilogram per day over 14 days or 20 mg. per kilogram per day over 7 days of the antibiotic in capsules by mouth. Clinically, within 3 to 8 days diarrhea was controlled, localized tenderness was relieved, and evidence of healed intestinal ulceration appeared in 4 to 10 days. Stools became negative for parasites in from 3 to 7 days. There were 4 failures among the first 10 cases treated, and 3 among the second 10, i.e., a failure rate of 7 out of 20. Blood, liver, and kidney function tests did not show appreciable changes. Side effects were limited to mild gastroenteric distress. While an unreported series by Carter (cited by Shafei) indicated success, when three stool examinations followed therapy, the detailed findings of this and other studies currently in progress are not yet available.* Shafei believes the "antibacterial effect may explain the more favorable results obtained in acute dysentery with concomitant bacterial infection when paromomycin is used."

References

1. Svanidze, D. P.: History of Amoebiasis and Measures Against It, Moscow, pp. 1-132, 1955.
2. Anderson, H. H.: The Use of Fumagillin in Amebiasis, Ann. New York Acad. Sc. **55**:1118-1124, 1952.
3. Phillips, B., and Wolfe, P. A.: Studies in Amoebiasis, Ann. New York Acad. Sc. **78**:308-314, 1959.
4. Adams, A. R. D.: Amoebiasis With Special Reference to Treatment, Tr. Roy. Soc. Trop. Med. & Hyg. **38**:237-258, 1944.
5. Wilmot, A. J., Armstrong, T. G., and Elsdon-Dew, R.: The Comparative Value of Amoebacidal Drugs, J. Trop. Med. **54**:161-165, 1953.
6. Kean, B. H.: Choice of Therapy in Intestinal Parasitic Disease, Cornell Conferences on Therapy, New York, 1955, The Macmillan Company, vol. 7, pp. 121-139.
7. American Drug Index, 1957, Philadelphia, J. B. Lippincott Company, pp. 544.
8. Nelson, T. L., Anderson, H. H., and Roh, J.: Erythromycin Therapy of Amebiasis in Children, in Welch, H., and Marti-Ibañez, F., editors: Antibiotics Annual 1954-1955, New York, 1955, Medical Encyclopedia, Inc., pp. 250-253.
9. Brooke, M. M., Heeren, R. H., Sawyer, G. M., and Stoner, D.: Mass Therapy in Attempted Control of Amebiasis, Pub. Health Rep. **73**:499-510, 1958.
10. Radke, R. A., and Baroody, W. G.: Carbarsone Toxicity: a Review of the Literature and Report of 45 Cases, Ann. Int. Med. **47**:418-427, 1957.
11. Browne, D. C., McHardy, G., and Edwards, E. W.: Amebiasis, a Clinical Evaluation, New Orleans M. & S. J. **102**:475-481, 1950.
12. Silva, R., Donckaster, R., and Valencia, R.: Study of Some Epidemiological Factors Favoring Infection and Re-infection by *E. histolytica*, Bol. chileno de parasitol. **13**:22-25, 1958.
13. Tobie, J. E., Most, H., Reardon, L. V., and Bozicevich, J.: Laboratory Results on Efficacy of Terramycin, Aureomycin and Bacitracin in Treatment of Asymptomatic Amebiasis, Am. J. Trop. Med. **31**:414-419, 1951.
14. Berberian, D. A., Dennis, E. W., Korns, R. F., and Angelo, C. A.: Drug Prophylaxis of Amebiasis, J.A.M.A. **148**:700-704, 1952.

*Wilmot, A. J.: Personal communi-

*Thompson, Paul: Personal communication.

15. Prophylaxis in Amebiasis. Questions and Answers, J.A.M.A. **166**:430, 1958.
16. McPhee, H. R.: Letter to the Editor, Mil. Med. **118**:236, 1956.
17. Kean, B. H., and Waters, S. R.: The Diarrhea of Travellers, New England J. Med. **261**:71-74, 1959.
18. Donckaster, R., Donoso, F., Martinez, P., and Thumala, A.: Effect of Pontalin (WIN 5047) in Chronic Intestinal Amebiasis, Bol. chilenc de parisitol. **14**:2-4, 1959.
19. Woodruff, A. W., Bell, S., and Schofield, F. D.: Treatment of Amoebiasis With Emetine, Glaucarubin Dichloroacet-hydroxy-methyl-anilide, Camoform and Various Antibiotics, Tr. Roy. Soc. Trop. Med. & Hyg. **50**:114-138, 1956.
20. de Carneri, Ivo: Use of Specific Antiamoebic Drugs for Comparative Taxonomic Studies, Tr. Roy. Soc. Trop. Med. & Hyg. **53**:120-121, 1959.
21. Mehta, S. P., et al.: Amebiasis, J. Indian M. A. **32**:March, 1959 as abstracted in Foreign Letters, J.A.M.A. **170**:993, 1959.
22. Sen, P., and Sanyal, N.: Report in Bull. Calcutta School of Trop. Med. **4**:81, 1956; Sen, P. et al.: Ibid **3**:75, 1955.
23. Chatterjee, P. K., Mukherjee, S., Ghose, S., Datta, K. P., and Sircar, S.: Clinical Trial With Two New Synthetic Compounds in Amoebiasis, J. Indian M. A. **30**:251-254, 1958.
24. Chowdhury, A. K. Roy, and Nandi, R.: New Synthetic Compounds in Treatment of Amoebiasis, J. Indian M. A. **30**:239-251, 1958.
25. Nagaty, H. F., Rifaat, M. A., Salem, S., and Khalil, H. M.: Clinical Trials of Compound 11925-C, Am. J. Trop. Med. & Hyg. **7**:416-418, 1958.
26. Angioni, G.: Nota preliminare sul trattamento dell'amebiasi intestinale con derivati chinonfenantrolinici (Entobex), Minerva Med. **49**:2463-2467, 1958.
27. Amaral, A. D. F., and de Avila Pires, C. D.: Observações sobre novo amebicida de largo espectro, Hospital **54**:75, 1958.
28. Beppler, W. A., and Schweiger, L. G.: Amebacidal and Trypanocidal Activity of a New Series of Diamines, Antibiotics & Chemother. **7**:513-520, 1957.
29. Anderson, H. H., Kwee, T. B., and Nelson, T. L.: Initial Trial of MA-307 in Chronic Human Amoebiasis, Arch. internat. pharmacodyn. **117**:146-150, 1958.
30. Anderson, H. H., Wilmot, A. J., Freedman, L., and Anderson, J. Van D.: Acute African Amoebiasis, Am. J. Trop. Med. & Hyg. **7**:201-204, 1958.
31. Shafei, A. Z.: The Treatment of Amebic Dysentery With Paromomycin, Antibiotic Med. & Clin. Therap. **6**:275-278, 1959.

The actions and uses of anthelmintics

The recent introduction of piperazine for the treatment of ascariasis and enterobiasis and dithiazanine for the treatment of trichuriasis, strongyloidiasis, ascariasis, and enterobiasis represent marked advances in helminth chemotherapy. The remarkable efficacy of the single-dose treatment of enterobiasis with pyrvinium pamoate is outstanding. There are also a number of new, promising compounds which require further critical clinical evaluation. Therapy of trichiniasis, clonorchiasis, paragonimiasis, filariasis, and infections with the dwarf tapeworm are unsatisfactory and require additional study. Gradually the physiology, and especially the metabolic patterns, of helminths are becoming unraveled and, in time, we may have a rational approach to helminth chemotherapy.

Harold W. Brown, M.D. *New York, N. Y.*
College of Physicians and Surgeons, Columbia University

Introduction

In his world-wide survey of helminth parasites of man, Stoll[48] came to the startling conclusion that the number of helminthic infections in the United States and Canada was 44,900,000 and in the world, 2,257,100,000. The latter number is greater than the total population of the world at the time of the survey and illustrates the principle that wormy people frequently harbor several different species of helminths at the same time. It is estimated that over 800,000,000 persons the world over are infected with helminths. In underdeveloped countries, the burden of helminth infection, added to that of malnutrition, bacterial, protozoal, and viral infections, may be the proverbial back-breaking straw. Well-nourished persons often harbor helminths without apparent damage and one may question the wisdom of treating such infections, especially with chemotherapeutic agents with toxic qualities. On the other hand, if a safe and effective drug is available, treatment should be given, until evidence is in hand that these helminth infections are beneficial to man. Although pinworm frequently causes man little or no inconvenience, and light infections of other worms may go unnoticed, schistosomiasis is a debilitating and deadly infection, heavy infection with several other helminths may cause very serious anemia, and the normal and aberrant wanderings of several species may be fatal.

Stimulated by the tropical campaigns of World War II, research workers here and abroad have continued to focus their attention on studies of parasitic diseases. As a result of the extensive chemotherapeutic studies, considerable progress has been made and a number of new agents have been developed. Although safety and efficacy are prime requisites, cheapness is also important, for, in general, the "wormy" are

often in the lowest economic group of a poor country. Frequently, the whole family requires therapy, not only for one species of worm, but for several types. Further, they will return in several months for treatment of their reinfection. A liquid, oral therapeutic agent is always advantageous, for ease in treating children, who bear the weight of the world's worm burden. Intravenous and intramuscular medication has great drawbacks, especially if repeated injections are required.

It is often difficult to evaluate the reputed efficacy of anthelmintics. Frequently, not only do dosages vary but, what is equally important, the criteria for reduction and elimination of the worm burden are dissimilar. Pretreatment and posttreatment laboratory examinations should be standardized. Egg counts are of great value and add greatly to the picture given by the cure rate. The cure rate depends on the refinement of the technique employed. Recovery of worms after therapy is valuable, for it confirms quantitative stool examinations for eggs and, further, it gives information on the condition of the expelled worm. Examination of the stool for eggs must be done sufficiently long after chemotherapy to permit injured worms to recover their egg-laying ability and still not permit sufficient time for a reinfection to occur.

Although many helminths superficially resemble each other, they often differ markedly in their reaction to chemotherapeutic agents, which indicates physiologic and biochemical differences. It must be remembered, however, that the habitat of the parasite often greatly affects the amount of contact the drug or its metabolites may have. Worms that are highly susceptible to a drug may be protected from its action by their habitat far down the intestine or by their embedded position in the tissues. Maintaining lethal concentrations of drugs in tissues, destructive to such parasites as Filaria and Schistosoma, is extremely difficult, especially if the host is to survive.

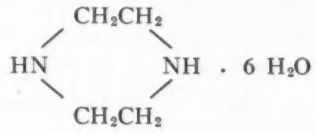
Most of the anthelmintics in use today were discovered empirically and, with few exceptions, their mode of action is unknown. The early studies of Bruening and Trendelenburg suggest that santonin and oil of chenopodium cause muscular stimulation followed by paralysis. Straub found aspidium to be fatal to invertebrates in general, as a result of muscular paralysis. While it is evident that tapeworms, which have no digestive tract, can be affected only through their external surface, it is not known in the case of other worms whether anthelmintics act after ingestion by the worm or after absorption through the body wall.[4] The exact mechanism of action of anthelmintics requires a more complete understanding of helminth physiology than is available at present. Extensive metabolic studies indicate that enzyme systems of helminths vary considerably and the careful work of Bueding and his colleagues[8] indicates that some anthelmintics are effective through their action on the various enzymes.

Enterobiasis, oxyuriasis

Enterobius vermicularis, pinworm, lives its short life of a month or two in the large intestine of man. The female is 8 to 12 mm. in length and the seldom-seen male is 2 to 5 mm. in length. Eggs are liberated by the gravid female when she migrates to the perianal region of the host. Infective eggs, after ingestion, develop into gravid female worms in 2 to 4 weeks. Throughout the world an estimated 208,800,000 persons are infected; in the United States and Canada, 18,000,000 persons. Diagnosis is made by microscopic demonstration of Enterobius eggs on a cellulose adhesive-tape swab of the perianal area of the patient.

The treatment of an individual harboring pinworm is frequently unrewarding if other infected members of the household are untreated and serve as a source of reinfection. It is recommended, therefore, that all members of the household be examined by the Scotch-tape swab technique and that those infected be treated simultaneously.

Drug of choice. The piperazine salts, citrate,* phosphate,† or adipate,‡ are the drugs of choice in enterobiasis; they are available as a palatable syrup, tablets, or wafers to be chewed.



Piperazine hexahydrate

In solution all of these compounds form piperazine hexahydrate and dosage is based on this compound. Hundreds of piperazine compounds have been tested against pinworms in mice but none of them proved superior to the three salts named above.[6] Piperazine is most effective against pinworm when taken as a single daily dose before breakfast and followed by a glass of water which carries the drug to the cecum and colon, the habitat of the worm. Therapy is continued for 7 consecutive days. Employing the following dosage schedule, the author[7] cured 58 of 60 patients.

Daily dose of piperazine used in therapy of enterobiasis

Patient's weight (pounds)	*Piperazine citrate syrup (ml.)*	*Piperazine hexahydrate (Gm.)*
Up to 15	2.5	0.25
16–30	5.0	0.50
31–60	10.0	1.0
Over 60	20.0	2.0

The majority of the pinworms are passed alive and active during the first 4 days of therapy.

Extensive experience with piperazine citrate in varying dosages and periods of therapy indicates that it is a safe anthelmintic. Its LD_{50} in mice is 11 Gm. per kilogram body weight. Side effects are very infrequent and consist of nausea, vomiting, headache, abdominal cramps, and, very rarely, urticaria. Excessive dosage has resulted in transient neurological disturbances, characterized by vertigo, tremor, incoordination, visual disturbances, sense of detachment, muscular weakness, and memory defects. Combes and associates[14] reported neurological side effects in a patient with chronic nephritis after 5 days of standard therapy, 2.0 Gm. of the hexahydrate (30 mg. per kilogram) daily. Failure to excrete the piperazine presumably led to a high concentration in the blood and tissues. Whether the presence of uremia, with its heightened reactivity of the nervous system, made the patient more susceptible to piperazine is problematical. Symptoms disappeared within a few days after the drug was discontinued.

Other drugs. Several cyanine dyes, pyrvinium chloride* and dithiazanine,† are highly effective against pinworm. However, as the course of therapy is approximately the same length as that of piperazine and as nausea, vomiting, and abdominal distress are relatively frequent, they offer no advantages over piperazine against pinworm. As dithiazanine is effective against several helminths, however, its use in multiple worm infections is an advantage.

Recently, Beck and co-workers[2] demonstrated that a single dose of pyrvinium pamoate, 5 mg. per kilogram body weight, cured 96 of 100 pinworm infections. The raspberry-flavored syrup was well received and none of the patients complained of side effects. Confirmation of these results is needed, but it appears that this drug may usher in a new era in pinworm therapy.

Gentian violet in enteric-coated tablets is an effective, inexpensive therapeutic agent for the treatment of enterobiasis. Therapy for 10 consecutive days is effective and relatively well tolerated, although nausea, vomiting, and abdominal pain are not uncommon. Recently, a 7 day course of therapy was reported as successful. The usual dose of gentian violet for adults is 65 mg. 3 times a day before meals.

*Antepar.
†Antepar, Piperazate.
‡Entacyl.

*Vanquin.
†Delvex, Abminthic.

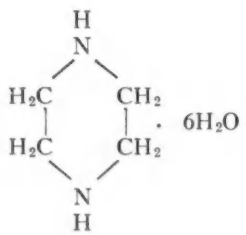
Oxytetracycline is effective in enterobiasis but expensive, especially when the treatment of a whole family is necessary. The usual dose for adults is 2.0 Gm. a day for 5 to 7 days.

The one-dose treatment with promethazine,* when adequately evaluated, has only its toxicity and lack of efficacy to recommend it.

The prevalence of this persistent parasite, both in the underprivileged and also in the seats of the mighty, has led to extensive chemotherapeutic studies and, over the years, the following compounds have been shown to be of some value but, from cost, bulk, side effects, and only moderate efficacy, are not practical: bacitracin and succinylsulfathiazole,† tetrachlorethylene, benzylphenyl carbamate, hexylresorcinol, thymyl-N-isoamylcarbamate, pyrathiazine hydrochloride, phthalylsulfathiazole and puromycin.

Ascariasis

Ascaris lumbricoides, the large roundworm, lives in the small intestine of man, feeding on its contents. It may reach a length of 35 cm. The female worm passes 200,000 eggs per day which makes diagnosis by stool examination easy and accurate. After 3 weeks in the soil, these eggs are infective when swallowed by man. In 2½ months, the worms grow to maturity and they live 12 to 18 months. World-wide infection is present in 644,000,000 persons; in the United States, 3,000,000 persons are infected.



Piperazine hexahydrate

Drug of choice. The use of piperazine in ascariasis by Fayard[18] was a significant advance. Piperazine citrate* and other salts are very effective and are prepared in palatable syrups, tablets, or chewable wafers which make for ease in administration to children. A single dose cures 55 to 85 per cent of the infections; therapy on 2 consecutive days will eliminate approximately 95 per cent. The cure rate of light infections is considerably higher than that of heavy infections.

Dosage schedule for one- and two-day treatment of ascariasis with piperazine

Patient's weight (pounds)	*Daily dose as citrate syrup (ml.)*	*Daily dose as hexahydrate (Gm.)*
30–50	20	2.0
51–100	30	3.0
Over 101	35	3.5

The piperazines may be given at any time, as the presence of food in the digestive tract has little, if any, effect on their activity against Ascaris. Special dietary precautions are unnecessary and purgation is not required.

The ascarides usually are passed on the first, second, or third day after treatment and are immobile and relaxed, though not dead. If they are placed in saline at 37° C., they regain their normal muscular activity. In vitro studies indicate that solutions of piperazine produce a state of relaxation of the worms after a few hours. The studies of Norton and de Beer[37] suggest that piperazine is a myoneural blocking agent, selective for Ascaris muscle, blocking the stimulating effects of acetylcholine. The paralyzed worms are unable to maintain their position in the host's intestine and are expelled by peristaltic action. Bueding[9] has suggested an additional mechanism, for he found that during incubation of Ascaris with piperazine, production of succinate is reduced, which may be concerned with the supply of energy required by the parasite for muscular contraction.

The paralyzing property of piperazine against Ascaris has been utilized by Jenkins and Beach[27] in the successful conservative

*Phenergan.
†Sulfasuxidine.

*Antepar.

treatment of partial intestinal obstruction due to this worm. The propensity of Ascaris adults to migrate to undesired locations or perforate the intestine of patients with high fever makes successful treatment of 36 patients with typhoid fever, who also harbored Ascaris, of special significance.[39] The flaccid worms are passed without danger of intestinal perforation.

Other drugs. Hexylresorcinol is an effective, somewhat expensive, ascaricide and has been used extensively in the past 30 years.[3] It is essential that the intestinal tract be empty when the drug is given, or it will combine with food and have little action on the parasite. Hexylresorcinol* is available only in pill form. The pills should be swallowed whole, for if they are chewed they cause a superficial, annoying burn. This drug produces necrosis and "blisters" on contact with the worm; it also changes the body wall's permeability to phosphorus compounds, which escape from the worm.

Diethylcarbamazine,† which contains the piperazine ring, is effective if given for 4 to 7 days. Oil of chenopodium and santonin are not as effective in safe doses as piperazine and occasionally may be very toxic. Because of its cheapness, however, chenopodium is still widely used in underdeveloped countries.

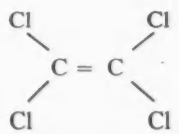
When Ascaris is accompanied by Trichuris or Strongyloides, dithiazanine, which is the drug of choice for the latter two worms, will also remove the Ascaris. When Ascaris is present alone, however, the effectiveness and absence of side effects of piperazine indicate its use rather than dithiazanine.

Bephenium hydroxynaphthoate has recently been shown to be effective in ascariasis and, in view of its efficacy against hookworm, it may become a drug of choice for these infections, especially when they are present together. The Ascaris egg count reduction in children given two doses of 2.5 Gm. (base) on one day was 91 per cent; all of the worms were removed from 13 of the 20 children treated.*

*Crystoids.
†Hetrazan.

Hookworm

Necator americanus and *Ancylostoma duodenale* live attached to the small intestine of man, sucking blood for nourishment. They are approximately 1.0 cm. in length. Eggs are passed in the host's stool. Larvae hatch from these eggs in the soil and they actively penetrate the skin of man. Although most adults with hookworm live only 2 to 5 years, they may live as long as 14 years. Each worm consumes from 0.026 ml. to 0.22 ml. of blood per day. The estimated number of hookworm-infected individuals in the world is 456,800,000 and in the United States, 1,800,000.



Tetrachlorethylene

Drug of choice. Tetrachlorethylene, introduced by Hall, has been the drug of choice for over 30 years. Tetrachlorethylene is safe, effective, cheap, liquid, and administered orally, an ideal anthelmintic. It replaced its relative, carbon tetrachloride, which is highly hepatotoxic. The recent studies of Carr and associates[12] have demonstrated that the standard dose of 3.0 ml., which was employed for 25 years and given either with a saline purge or followed 2 hours later by a purge, was not optimal. They observed that when larger amounts of tetrachlorethylene were administered without concomitant or subsequent purgation, the patients experienced fewer side effects and the chemotherapeutic results were superior. From careful studies on over 50,000 Necator-infected patients, they demonstrated that a single dose of 5.0 ml. of tetrachlorethylene is entirely safe and will remove approximately 92 per cent of the worms. They gave children 0.05 to 0.06 ml.

*Hsieh, Brown, Fite, Chow, Cheng, and Hsu. In manuscript.

of the drug per pound of weight, to a maximum dose of 5.0 ml. A light meal is eaten the night preceding treatment and no breakfast the following morning. Tetrachlorethylene is given at 7 to 8 A.M. and a noon meal, relatively fat free, may be eaten. Several treatments are often required to remove all of the hookworms. Patients with severe hookworm anemia, with as little as 1.5 Gm. of hemoglobin per 100 ml. of blood, were treated without serious side reactions when purgation was not employed.

Ancylostoma duodenale appears to be considerably more difficult to remove than *Necator americanus*. Hill and Andrews[24] and others have demonstrated the great value of iron therapy in severe hookworm infections. Hemoglobin increased from 2.0 to 2.5 Gm. to 9 to 10 Gm. after 4 to 5 weeks of iron therapy alone.

Tetrachlorethylene is quite water insoluble, 1 part in 10,000, and in the absence of fat in the intestine it is not absorbed to any extent. When given to animals in high dosage, there is no evidence of hepatic or renal damage. Liver function tests on man are normal during therapy with this drug. It has been given to millions of patients, many of them greatly debilitated, with only occasional nausea, vomiting, giddiness, mild inebriation, and in two instances, temporary loss of consciousness.

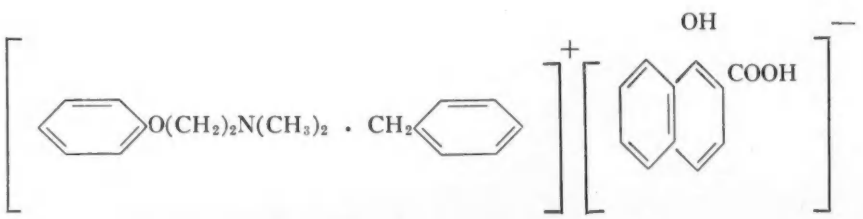
In patients harboring both Ascaris and hookworm, the Ascaris is usually removed first with a suitable anthelmintic before tetrachlorethylene therapy is employed for hookworm. This procedure is based upon numerous observations that carbon tetrachloride stimulates the adult Ascaris to migrate within the host, often with serious consequences. This fact has been carried over to the close relative tetrachlorethylene, but without adequate scientific or clinical evidence. Extensive groups of hookworm-infected patients have been treated with this drug without complications from the concurrent Ascaris infection. Some authors have even noted the modest efficacy of tetrachlorethylene against Ascaris.

The mode of action of tetrachlorethylene is unknown; although water insoluble, it is very soluble in fat. Mueller[35] has called attention to the fat deposits in the hypoderm muscle cells, especially around their nuclei, in the processes passing from the muscle cells to the nerve trunks, and in the lateral lines close to the main nerve trunks of Ascaris. He suggests these areas as possible sites of action of halogenated hydrocarbon compounds against nematodes.

Other drugs. Hexylresorcinol is moderately effective against hookworm and, of course, has the added advantage of its great activity against Ascaris. Because, however, of its cost, pill form, and the deleterious effect of food on its efficacy, it is recommended for hookworm infection only under special conditions.

Cyanine dyes, which have been tested, have proved to be only moderately effective against *Necator americanus* and, in the dosage employed, have produced uncomfortable side effects. Dithiazanine therapy gives good egg-count reductions in some infections but the cure rate is low.[19]

A group of quaternary ammonium compounds have recently been shown to have activity against a wide range of nematodes in laboratory and domestic animals and man.[20]



Bephenium hydroxynaphthoate

One of them, bephenium hydroxynaphthoate* shows considerable promise against Necator, Ancylostoma, and Ascaris and some effect against Trichuris.[21] Hsieh and the author have confirmed these studies, utilizing a total daily dose of 5.0 Gm. (base) in children. Slight nausea, vomiting, and soft stools were observed after therapeutic amounts of the hydroxynaphthoate. Studies by Rogers[42] show that only a very small amount of the drug and its metabolites is excreted in the urine.

Betanaphthol was one of the early hookworm remedies. Recently trials with 1-bromo-β-naphthol indicate it has significant activity against both Necator and Ancylostoma in man.[36] Likewise, further evaluation of N-(2,4-dichlorobenzyl)-N-(2-hydroxyethyl) dichloroacetamide† seems indicated in view of its reported efficacy.[44]

Trichuriasis

Trichuris trichiura, the whipworm of man, infects 355,000,000 persons in the world and 400,000 persons in the Southern United States. The adult worms are 30 to 50 mm. in length and they live with their anterior end embedded in the mucosa of the cecum and large intestine. Numerous eggs passed in man's stool incubate in the soil, serve as a source of infection when ingested. The worm lives many years and when present in considerable numbers causes a severe anemia. Diagnosis is made by the presence of the characteristic barrel-shaped eggs in the stool.

*Alcopar.
†Win 5047, Mantomide.

Drug of choice. Because of its position in the cecum and large intestine, Trichuris has been difficult to eliminate by oral therapy. Until recently, enemas containing hexylresorcinal have given the best therapeutic results against this parasite. The discovery of the therapeutic activity of dithiazanine, 3,3′-diethylthiadicarbocyanine iodide, by McCowen and his colleagues[33] in 1957, made available for the first time a safe and effective agent for the oral therapy of trichuriasis.

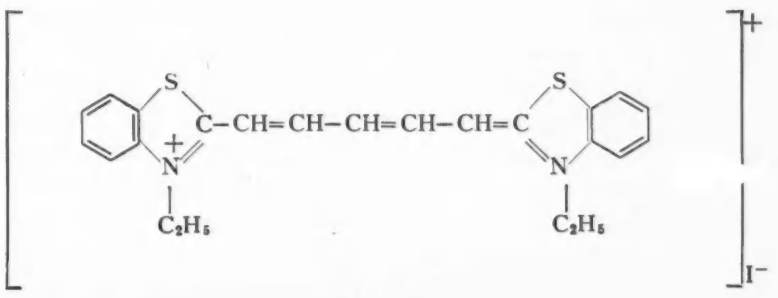
Frye and co-workers[19] reported a cure rate, based on Stoll dilution egg counts, of 72 per cent following dithiazanine three times a day for 5 days. Clinical cure of heavy whipworm infections with dithiazanine is striking and the dysentery which is sometimes present quickly subsides. Iron therapy is indicated for the severe anemia that is often present in severe Trichuris infections.

Total daily dosage of dithiazanine, 5 day therapy

	Patient's weight (pounds)				
	20-30	30-40	40-50	50-60	Over 60
Daily dose	200 mg.	300 mg.	400 mg.	500 mg.	600 mg.

In children, daily dosages may be divided into two doses if three doses are impractical.

Employing the above dosages, we found that a single course of dithiazanine eliminated all of the whipworms from 60 per



Dithiazanine

cent of 34 patients, based upon stool examinations by the sedimentation technique. The worms were dead and some were green-stained when passed. However, all patients benefit from therapy as it has been shown that the average egg-count reduction following one course of therapy may be as great as 98.2 per cent. Several courses of dithiazanine may be necessary to eliminate whipworm completely. Vomiting occurred in 40 per cent of our patients, often only after the first or second dose of the drug, but sometimes after each dose. Only rarely was therapy stopped because of the severity of the vomiting. In view of the longevity of Trichuris and the loss of blood due to it, the minor side reactions seem unimportant.

Studies[40] demonstrate that cyanine dyes produce an inhibition of the oxygen uptake of adult *Litomosoides carinii*, a filarial worm of the cotton rat. This respiratory inhibition is associated with a compensatory increase in aerobic glycolysis. It is suggested that cyanines exert their chemotherapeutic effect through inhibition of enzymes concerned with oxidative metabolism. The biochemical action of cyanines on *Trichuris vulpis* of the dog indicates their effect is not limited to oxidative processes, but may involve anaerobic metabolism as well. Dithiazanine apparently affects the reproduction mechanism of Trichuris, for following therapy deformed eggs are present in the stools and in the dissected worms.

Other drugs. With the exception of dithiazanine, the treatment of Trichuris infections by means of anthelmintics administered orally is only moderately successful. The author[3] gave 368 Trichuris infected patients a single treatment with hexylresorcinol pills and reduced the average egg count 32 per cent. Retention enemas with hexylresorcinol are effective, but they are too time consuming for mass therapy and their use was confined largely to heavily infected anemic patients. Hoekenga[26] cured 50 per cent of his trichuriasis patients with a single dose of a mixture of tetrachlorethylene and oil of chenopodium.

Piperazine citrate is not effective against Trichuris. Several authors[17] report good results from the use of piperazine adipate in whipworm infections. Other workers,[26] including the author, failed to secure significant therapeutic effects from this drug.

Other drugs. 3-Methyl-1-pentyn-3-yl acid phthalate* is effective against the canine whipworm. However, in the dosage found effective in man, temporary conjunctivitis, keratitis, photophobia, and ocular pain were produced.[26]

Leche de higuerón, the sap of the fig tree of the genus Ficus, according to the Caldwells,[11] will remove 85 per cent of the Trichuris of man. Robbins[41] demonstrated this sap to contain a proteolytic enzyme which has a specific action against Trichuris and Ascaris, yet does not digest the intact intestinal mucosa of their host. Unfortunately, leche de higuerón deteriorates with age and the comparatively large dose is quite unpleasant to take.

Shrapnel[45] observed that during oral emetine hydrochloride therapy of amebiasis there was a noticeable effect on the Trichuris of several of his patients. Following this lead, Burrows, Morehouse, and Freed[10] treated Trichuris-infected adults with varying amounts of "enteric-sealed" emetine hydrochloride tablets. There was an 88 per cent reduction in Trichuris egg count following treatment, and large numbers of dead worms were passed in the stools, in one instance 2,538. Diarrhea was experienced by all of the patients while under treatment, and nausea and vomiting were also present to a lesser extent. When the diarrhea was at its height, sloughed intestinal mucosa with dead worms attached and blood were found in the stools. It is apparent that emetine hydrochloride has considerable activity against Trichuris, but because of its toxicity and injury to the host's tissues it cannot be recommended.

Preliminary studies indicate that bephenium hydroxynaphthoate is moderately effective against Trichuris. Further clinical trials may lead to a more effective dosage schedule.

*Whipcide, Phtholofyne.

Strongyloidiasis

The parasitic female generation of *Strongyloides stercoralis* lives embedded in the mucosa of the duodenum and upper jejunum of man. The adult female worm is only 2.2 mm. in length. It passes eggs into the intestinal mucosa which develop into larvae before they are passed in the stool. Infection may occur as in hookworm, by larvae in the soil penetrating the skin of man. Autoinfection by larvae in the intestinal tract may occur and this explains the heavy infections found as long as 36 years after possible infection from the soil. Stoll estimates that 34,900,000 persons in the world and 400,000 in the United States are infected with Strongyloides. Diagnosis is made by finding the microscopic larvae in the stool.

Drug of choice. The position of the parasitic female, embedded in the intestinal mucosa, renders therapy difficult. Careful laboratory and clinical studies during the past 2 years have demonstrated the efficacy of cyanine dyes, pyrvinium chloride* and dithiazanine iodide,† against strongyloidiasis.[52] Both compounds in therapeutic amounts not infrequently cause nausea, vomiting, abdominal distress, and anorexia. These minor side effects must be weighed against the cure of a long-lasting, sometimes debilitating, and even fatal infection. At present, the most satisfactory therapy for an adult consists of dithiazanine, 200 mg. 3 times a day for 10 to 14 days. (See Trichuris for children's doses.) This regimen will cure the majority of Strongyloides infections.

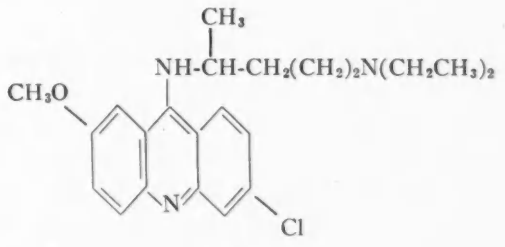
Careful studies of renal and hepatic functions and hematologic examinations, during and after therapy, revealed no significant alterations. It appears that we now have a safe and effective chemotherapeutic agent for strongyloidiasis.

Other drugs. Until recently, gentian violet given orally was widely used in strongyloidiasis. Although complete elimination of the infection was usually not achieved, clinical improvement was often noted. For adults, 0.065 Gm. 3 times a day, before meals, for 17 days is standard therapy. Nausea, vomiting, abdominal pain, and diarrhea or constipation not infrequently accompany this therapy.

A number of other drugs have been used against Strongyloides with indifferent or equivocal results: quinacrine, piperazine, promethazine,* hexylresorcinol, and lucanthone hydrochloride.†

Cestodiasis

Man commonly is host to three large tapeworms, *Taenia saginata, T. solium,* and *Diphyllobothrium latum,* acquired from eating the larval forms in beef, pork, and fish, respectively. Usually, only one worm is present, although multiple worms may be encountered. The dwarf tapeworm, *Hymenolepis nana,* is found in man, especially children, in great numbers, and is acquired by ingesting the eggs of the parasite passed in the host's stools or from insect intermediate hosts. Man also accidentally acquires several dog, cat, and rat tapeworms. All of these adult worms live attached by their heads to the small intestine of man. The world over, some 70,000,000 persons harbor these elongate parasites. The eggs of all of these worms are found in the stool and frequently the segments of the Taenia are also present.



Quinacrine

Drugs of choice. Although a number of new anthelmintics for cestode therapy have recently been reported, until confirmation of these studies has been made, quinacrine

*Vanquin.
†Delvex.

*Phenergan.
†Miracil D.

hydrochloride* remains as the safest, most effective therapeutic agent available at present for the treatment of tapeworm infection.[46] Following therapy with quinacrine, the worms are usually alive when passed and are often stained bright yellow. The mode of action of quinacrine is unknown. As the strobila of the worm is regenerated from the head (scolex) and neck, effective therapy must remove these portions of the parasite. This involves freeing the intestine of food which would keep the drug from coming in contact with this small but vital portion of the tapeworm. The preparation of the patient is therefore important in tapeworm therapy. On the day preceding treatment, the patient should be placed on a liquid diet and supper may be omitted except for black coffee, tea, and water. A soapsuds enema should be given in the evening. The following morning the patient should remain in bed, omit breakfast, and take the anthelmintic. The total dose of quinacrine for adults is 0.8 Gm. In order to prevent vomiting, it may be given as 2 doses of 0.4 Gm. half an hour apart, or 0.1 Gm. every 10 minutes. Children's dose is 0.4 Gm. for children weighing 40 to 75 pounds, 0.6 Gm. for children 76 to 100 pounds.

Two hours after the last dose of the drug, a saline purge is administered, followed by a glass of water. If the worm is not removed by purgation or if the head is not recovered, a soapsuds enema should be given as the worm or scolex may be lodged in the large intestine. Toilet paper used by the patient should not be placed in the bedpan as it makes the search for the tapeworm head very difficult. If large portions of the worm are recovered and the head is not found, the stool should be free of eggs and segments for 6 months before one can be sure the treatment was successful. The head may have been digested, lodged in the large intestine, or not recognized in the stool examination following therapy and, therefore, failure to find the head does not necessarily mean failure to cure.

In the dosage employed in tapeworm therapy, quinacrine may produce nausea, vomiting, and occasionally transient dizziness and yellowish discoloration of the skin. Vomiting is potentially dangerous in patients harboring *Taenia solium* as it may result in segments and eggs being regurgitated into the stomach with the production of cysticercosis. Vomiting also reduces the efficacy of quinacrine against *T. saginata* and *D. latum*. We have found that chlorpromazine* administered prior to therapy is effective in reducing nausea and vomiting. Patients, either adults or children, who vomit quinacrine may be successfully treated with medication given by duodenal tube.

Other drugs. Galen used male fern, oleoresin of aspidium, as an anthelmintic. It is an extract of the green rhizome and stipes of *Dryopteris filix-mas* and contains many constituents. Anttonen[1] maintains that the active principle of extract of male fern is flavaspidic acid with which he cured 65 of 76 tapeworm infections. Other workers consider filicic acid to be the most active constituent of aspidium. Magath and Brown[30] report success with an emulsion consisting of 6.0 Gm. or 6.0 ml. of oleoresin of aspidium (the author uses 5.0 ml.), 8.0 Gm. of acacia and water to make up to 60 ml.; 30 ml. of the emulsion is given early in the morning, followed by an additional 30 ml. 1 hour later. The total dose for children is 4.0 ml. of the emulsion per 10 pounds of weight. Therapeutic doses of oleoresin of aspidium may cause, in varying severity, headache, vertigo, gastroenteritis, abdominal pain, diarrhea, nausea, vomiting, and temporary disturbances in vision.

Recently, when the author failed to remove a Taenia or Diphyllobothrium with quinacrine therapy, he has given aspidium on the following day with 100 per cent success in 4 cases. The patient remains on a liquid diet the second day so the intestine is free of food and the worm may be somewhat weakened by the additional starvation. Other workers report that a repetition

*Atabrine.

*Thorazine.

of quinacrine on the second day also brings out recalcitrant cestodes.

Unfortunately, we do not have an effective chemotherapeutic agent against *H. nana*. This tapeworm is frequently present in large numbers, which makes its complete eradication very difficult.

Tin was used as a vermifuge in the days of Paracelsus. Following extensive use in England, Germany, and Italy, it fell into disrepute, possibly because of the toxicity of arsenic and lead present in the impure tin compounds of the day. Hirte,[25] using a stable tablet preparation called Cestodin, which contains metallic tin and also its oxide and chloride, cured 56 of 58 tapeworm infections. The medication was given for 5 days without toxic or undesirable side effects. Kuhls[28] extended and confirmed these results. His tin mixture also contains zinc chloride. He treated 202 persons, all of whom harbored *T. saginata* except 3 infected with *T. solium* and 2 with *D. latum*. One hundred and eighty persons were cured and side effects from the drug were negligible. Living, active tapeworm proglottides are passed on the third or fourth day of therapy, but the head is seldom found. It is supposed that the tin is toxic to the head and the budding neck zone and that these parts are destroyed by the digestive juices.

Several recent studies suggest that the 4-aminoquinoline, amodiaquin,* is highly effective against Taenia. van Grunderbeeck and Penson[53] treated 130 patients with taeniasis with good results and Lecuona[29] reported a 95.6 per cent cure rate using this compound. In view of these excellent results and the relative safety of the drug, additional studies are indicated.

2,2′-Methylene-bis(5-chlorophenol)† has been introduced from veterinary medicine to human subjects with some success for the treatment of Taenia.[32] Several instances of jaundice and one death indicate that this drug is potentially dangerous to man when the standard dose is exceeded.[26a]

*Camoquin.
†Di-phenthane 70, Anthiphen, Dichlorophen.

In view of the number of chemotherapeutic agents which have been used with success against cestodes, a careful trial should be made of all of them, with a standard procedure for assessing cure. Either the scolex should be passed in the stool, or the patient should be free of tapeworm for 4 to 6 months post treatment.

Trichiniasis

Stoll[48] estimated that throughout the world there are 27,800,000 persons infected with *Trichinella spiralis* and that 21,100,000 of them live in the United States and Canada. Of the 350,000 estimated new infections which develop in the United States each year, only 16,000 may be expected to exhibit clinical symptoms.

The adult *Trichinella spiralis* is a small, threadlike organism, the male measuring 1.6 mm. in length, and the female 4.0 mm. Man becomes infected by ingesting the live encysted larvae in raw or partially cooked pork and pork products. Bear and walrus steaks have been the cause of several epidemics. The ingested larvae excyst in the upper intestine and invade the duodenal and jejunal mucosa where they develop to maturity, and within 5 to 7 days the females begin to pass larvae. The larvae, approximately 100 μ in length, migrate by way of the lymphatics and mesenteric venules into the systemic circulation and are distributed throughout the body. Within 3 or 4 weeks, the female has passed most of her 1,500 progeny, although she may continue to produce them for as long as 16 weeks. The larvae have a predilection for striated muscle, especially the extraocular muscles, the diaphragm, larynx, base of the tongue, intercostal, pectoral, biceps, deltoid, gluteus, and gastrocnemius muscles. Approximately 3 weeks after infection, an ellipsoidal sheath is developed about the larvae by the degenerative products of the invaded muscle fiber. The encapsulated larvae in straited muscle grow to a length of 0.8 to 1.0 mm., and some of them remain viable for several years, then die and undergo calcification.

There is no satisfactory chemotherapeutic agent for use against the adult Trichinella in the intestinal wall or the larvae in the muscles. Several studies[16] have demonstrated that the administration of 200 mg. of ACTH per day to patients with trichiniasis produces marked clinical improvement in severe infections and may be lifesaving. Similar favorable results have been reported from the use of cortisone.[43] These two agents do not adversely affect the adult or larval Trichinella. In fact, Stoner and Godwin[49] found that cortisone-treated mice harbored more adult worms for a longer period of time and presumably pass more larvae than host mice not receiving cortisone therapy. These hormones, however, greatly reduce the hosts' tissue reaction to the invading larvae and produce striking clinical improvement in man. Unfortunately, the diagnosis of trichiniasis is usually made after the larval migration is already very extensive and the adult worms are embedded in the intestinal mucosa. Before the young adults are embedded, purgation or anthelmintic therapy would eliminate some of the parasites, thus decreasing the number of invading larvae. A great variety of agents has been tried against the adult worms in man, and some appeared to give clinical relief to individual patients. However, laboratory studies on experimentally infected animals did not confirm the therapeutic value of the agents. Over 140 different chemical compounds tested on experimental animals have failed to affect either the adult or the larval worms.

Recently, Chan and Brown[13] demonstrated that massive doses of piperazine markedly reduced the number of Trichinella adults in infected mice. They suggest that piperazine citrate, if employed during the diarrheal stage of the infection when the young adults are migrating into the intestinal wall, might eliminate some of them, thereby reducing the number of larvae invading the muscles. These workers recognize that the intestinal wall of mice is thinner than that of man and may not, therefore, afford as complete protection as does the thicker intestinal wall of man. Von Brand, Holtz, and Vogel[54] produced rapid calcification of Trichinella cysts in rats by oral administration of irradiated ergosterol; a dangerous parallel calcification of the host's organs was also noted, however.

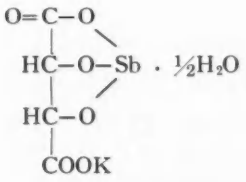
Schistosomiasis

Schistosomiasis is a chronic disease caused by the eggs and the adult schistosomes or blood flukes. *Schistosoma mansoni* and *S. japonicum* live in the small venules of the intestine and *S. haematobium* in the vesical and pelvic plexuses. The migration of the eggs through the intestine and urinary bladder and their accumulation in the liver, lungs, and central nervous system produce the characteristic syndrome.

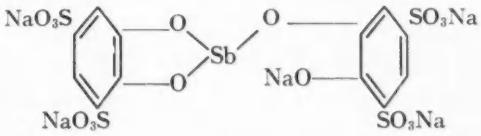
The adult worms are 0.6 to 2.5 cm. in length, and, depending upon the species of worm, from 300 to 3,500 eggs are passed daily into the venules. The eggs effect a passage into the lumen of the intestine or urinary bladder and are evacuated in the feces or the urine. On contact with fresh water, the miracidia hatch from the eggs and swim about until they find an appropriate snail, which they penetrate. After development and multiplication within the snail, the forked-tailed cercarial progeny emerge. During bathing, swimming, working, or washing clothes, the free-swimming cercariae penetrate the skin and migrate by way of the blood stream to their sites of predeliction. The adult worms live as long as 25 years. Approximately 125,000,000 persons harbor this parasite which causes a chronic, seriously debilitating disease. We see this infection not infrequently in our large New York Puerto Rican population. Fortunately, with adequate sewage disposal and lack of suitable snail hosts, it does not spread in the United States.

Drugs of choice. Trivalent antimony compounds, tartar emetic (potassium antimony tartrate), sodium antimonyl gluconate,* and stibophen† are the drugs of choice in schistosomiasis.[34]

*Triostam.
†Fuadin.



Antimony potassium
tartrate (tartar emetic)



Stibophen

Stibophen, though less effective than tartar emetic, is less toxic and is, therefore, preferred by many. Tartar emetic is slowly administered intravenously as a 0.5 per cent solution as follows: first day, 8 ml.; third day, 12 ml.; fifth day, 16 ml.; seventh day, 20 ml.; ninth day, 24 ml.; and on alternate days through the twenty-ninth day, 28 ml. (total 360 ml. containing 0.648 Gm. of antimony).

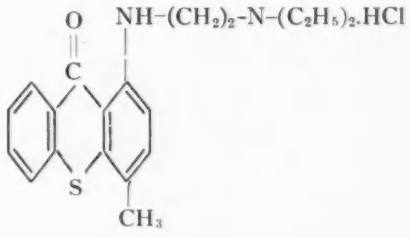
Stibophen is given intramuscularly as a 6.3 per cent solution. The course of treatment for adults consists of 20 injections of 5 ml. on alternate days, starting with 1.5 ml. and 3.5 ml. in the first two injections. Children are given a total dose of 1.0 ml. per pound of weight in 20 injections. Relapses occur not infrequently, so repeated stool examinations should be made several months after the completion of therapy and a second course of treatment given if indicated.

It has been generally assumed that the action of trivalent antimony compounds against parasites is similar to that of arsenicals, which inactivate the sulfhydryl groups of enzymes. Recent studies,[31] however, indicate that arsenic and antimony do not have identical antienzymatic spectra. One possible site of activity of trivalent antimony is the interference with a single step in the glycolysis of S. *Mansoni* by inhibition of phosphofructokinase. Pentavalent antimony compounds, although they have no schistosomal activity in vitro, are active in vivo, which suggest that they are reduced to the active trivalent form in the host, possibly in the liver.

Trivalent antimony compounds have an affinity for red blood cells, have a low blood plasma concentration, and are slowly excreted, for the most part by the kidneys. Little is known of the distribution of trivalent antimony in the tissues of man. In animals, high concentrations are found in the liver and thyroid.

Therapeutic use of antimony compounds may be attended by untoward reactions which are more frequent with trivalent than pentavalent compounds and more frequent with tartar emetic than other trivalent antimonial drugs. Severe coughing is associated with tartar emetic therapy. All trivalent compounds may produce nausea, vomiting, and joint pains. Headache, abdominal pain, mild rashes, and fainting may occur. Electrocardiographic changes are frequent but they are not associated with cardiovascular symptoms. The electrocardiogram reverts to normal 30 to 60 days after the cessation of therapy. Hepatitis is a rare but serious complication and renal damage is sometimes noted. Mass therapy with antimony compounds is not successful because of the necessity for repeated intravenous or intramuscular injections.

There is a great need for an orally effective chemotherapeutic agent and the thioxanthone, lucanthone hydrochloride,* represents a step in this direction. It is given at a rate of 10 to 20 mg. per kilogram of body weight for 8 to 20 days.[22]



Miracil D

Miracil D is effective against S. *haematobium*, less effective against S. *Mansoni*, and

*Miracil D, Nilodin.

of little value against S. *japonicum*. Side effects consist of nausea, vomiting, yellow discoloration of the skin, vertigo, restlessness, headache, mental confusion, and tremor. Children appear to tolerate the drug as well as, or even better than, adults. Miracil D is degraded in the body, presumably in the liver, with 7 per cent appearing in the urine.

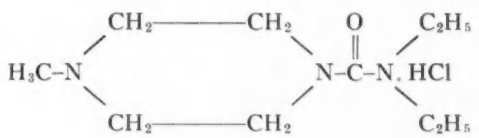
Other drugs. Standen[47] has studied a series of diphenoxyalkanes and has found a number of them to be active against S. *mansoni* and S. *japonicum* in animals. Affected schistosomes first lose muscle tone, are rendered immobile, and are swept into the liver. Here, a foreign-body reaction ensues, the worms become ensheathed in inflammatory tissue and are destroyed piecemeal. It is suggested that these compounds may exert their antischistosomal activity on the parasite and also stimulate the defense mechanism of the host, either directly or through the injured worm.

Filariasis

Man is host to 6 filarial worms all of which are characterized by their threadlike appearance, slow development, and long life. The adult worms live in the lymphatics, connective tissues, or body cavities. The female worm produces microfilariae which circulate in the blood or in the skin of the host. Some of the microfilariae are ingested by bloodsucking anthropods and, after undergoing development in them, they are deposited on the skin when this vector takes another blood meal and the infective larvae penetrate the skin and develop into adult worms.

Various domestic and wild animals harbor numerous species of filaria which are used for chemotherapeutic evaluation. Chemotherapeutic activity is ascertained during life by the reduction in number of microfilariae in the blood or skin snips, or, at autopsy, by the condition of the adult worms. Studies on man are usually limited to the effect of the drug on the circulating microfilariae for, except in onchocerciasis, the adult worms are practically impossible to find at biopsy. Occasionally, a chemotherapeutic agent kills the adult worm and the resultant inflammation about the dead and degenerating parasite gives qualitative evidence of the therapeutic efficacy.

Drug of choice. Diethylcarbamazine, 1-diethylcarbamyl-4-methylpiperazine,* is effective against several species of filaria. The usual dose is 2 mg. (citrate) per kilogram of body weight 3 times a day for 7 to 21 days. The LD_{50} in mice is 550 mg. per kilogram and in rats 395 mg. per kilogram when given orally.



Diethylcarbamazine

The piperazine ring is probably of fundamental importance in the activity of this compound although piperazine itself has no activity against filaria. The microfilariae of *Wuchereria bancrofti* and *W. malayi* disappear quickly from the blood after the beginning of therapy and, as they do not reappear on cessation of therapy, it is presumed that the adult worms are killed or at least they are permanently sterilized. After several courses of the drug, the adults of *Loa loa* are killed and also the microfilariae. In *Onchocerca volvulus* infections treated with diethylcarbamazine, the microfilariae in the skin are quickly killed and a severe allergic reaction often immediately ensues. Suramin† is effective against the adult Onchocerca, especially the female worms. However, as multiple injections are necessary and it is potentially toxic, it does not have wide application. Removal of the nodules containing the adult worms demonstrates that many of them have been killed or sterilized by the drug.

In cotton rats treated with diethylcarbamazine, the microfilariae are found concentrated in the liver. Within an hour, phago-

*Hetrazan, Banocide, Caricide, or Notezine.
†Naphuride, Bayer 205.

cytes surround these trapped microfilariae and within 18 hours, most of them have been destroyed.[23] These observations suggest that the drug sensitizes the microfilariae in some way so that they become susceptible to the action of the fixed macrophages. Man rapidly absorbs diethylcarbamazine and, when it is administered as a single dose orally, a peak blood level is reached in 3 hours. By utilizing the drug labeled with C^{14} in the piperazine ring, it has been found that rats and monkeys excreted about 70 per cent of piperazine metabolites in 24 hours. The drug is distributed throughout all organs, blood cells, tissues, and body fluids.

Other drugs. A number of antimony- and arsenic-containing compounds, given intramuscularly or intravenously, have been used with some success against filarial infections in man and animals. In general, the trivalent antimony compounds act rapidly on the microfilariae and more slowly on the adult worms.[5] In contrast, the pentavalent antimony compounds exert their effect on the adult worms and gradually the microfilariae disappear from the blood over a period of months because they are not replaced by the injured and dead adult females.[15]

Otto and Maren[38] found that arsenamide, a soluble thioglycollate derivative of benzamide *p*-arsenoxide, given intravenously, has a direct effect on both the microfilariae and adult worms. Unfortunately, it is hepatotoxic.

Although the antimony- and arsenic-containing compounds are interesting and they demonstrate that filaria can be attacked in its human host, these compounds all have the inherent defect of unpleasant side effects, even severe toxic reactions, and they all require multiple injections.

References

1. Anttonen, V. M.: Flavaspidic Acid as Anthelmintic, Ann. med. exper. et biol. fenniae **32**:15-25, 1954.
2. Beck, J. W., Saavedra, D., Antell, G., and Tejeiro, B.: Treatment of Pinworm Infections in Humans (Enterobiasis) With Pyrvinium Chloride and Pyrvinium Pamoate, Am. J. Trop. Med. **8**:349-352, 1959.
3. Brown, H. W.: The Treatment of Ascariasis and Trichuriasis With Hexylresorcinol Pills, Am. J. Hyg. **16**:602-608, 1932.
4. Brown, H. W.: Observations on the Mode of Action of Several Anthelmintics Upon Ascaris lumbricoides (Pig Strain), Am. J. Hyg. **26**:72-83, 1937.
5. Brown, H. W.: Treatment of Filariasis With Anthiomaline (Lithium Antimony Thiomalate), Ann. New York Acad. Sc. **50**:51-72, 1948.
6. Brown, H. W., Chan, K. F., and Hussey, K. L.: The Efficacy of Piperazine Compounds Against Syphacia obvelata, a Pinworm of Mice, Am. J. Trop. Med. **3**:504-510, 1954.
7. Brown, H. W., Chan, K. F., and Hussey, K. L.: Treatment of Enterobiasis and Ascariasis With Piperazine, J. A. M. A. **161**:515-520, 1956.
8. Bueding, E., and Most, H.: Helminths. Metabolism, Nutrition and Chemotherapy, Ann. Rev. Microbiol. **7**:295-326, 1953.
9. Bueding, E., and Swartzwelder, C.: Anthelmintics, Pharmacol. Rev. **9**:329-365, 1957.
10. Burrows, R. B., Morehouse, W. C., and Freed, J. E.: Treatment of Trichuriasis With Enseals of Emetine Hydrochloride, Am. J. Trop. Med. **27**:327-338, 1947.
11. Caldwell, F. C., and Caldwell, E. L.: A study of the Anthelmintic Efficacy of Higuerolatex in the Treatment of Trichuriasis, Am. J. Trop. Med. **9**:471-482, 1929.
12. Carr, P., Pichardo Sarda, M. E., and Aude Nunez, N.: Anthelmintic Treatment of Uncinariasis, Am. J. Trop. Med. **3**:495-503, 1954.
13. Chan, K. F., and Brown, H. W.: Treatment of Experimental Trichinosis in Mice With Piperazine Hydrochloride, Am. J. Trop. Med. **3**:746-749, 1954.
14. Combes, B., Damon, A., and Gottfried, E. Piperazine (Antepar) Neurotoxicity. Report of a Case Probably Due to Renal Insufficiency, New. England J. Med. **249**:223-224, 1956.
15. Culbertson, J. T.: Experimental Chemotherapy of Filariasis, Tr. Roy. Soc. Trop. Med. & Hyg. **41**:18-54, 1957.
16. Davis, W. M., and Most, H.: Trichinosis, Case Report With Observations of the Effect of Adrenocorticotropic Hormone, Am. J. Med. **11**:639-644, 1951.
17. Dunn, T. L.: Effect of Piperazine Derivatives on Certain Intestinal Helminths, Lancet **1**:592-593, 1955.
18. Fayard, C.: Ascaridiose et piperazine. Thesis, Paris, 1949. (Quoted from Semaine Hôp. Paris **35**:1778, 1949.)
19. Frye, W. W., Swartzwelder, C., Lampert, R., Abadie, S. H., and Carson, C. B., Jr.: An Ef-

fective Trichuricide Suitable for Oral Administration, Am. J. Trop. Med. **6**:890-893, 1957.

20. Goodwin, L. G., Jayewardene, L. G., and Standen, O. D.: Clinical Trials With Bephenium Hydroxynaphthoate (Alcopar) Against Hookworm in Ceylon, Brit. M. J. **2**:1572-1576, 1958.
21. Gunders, A. E.: Clinical Trials With Bephenium Hydroxynaphthoate (Alcopar) Against Intestinal Nematodes in Liberia. (Submitted to Am. J. Trop. Med. & Hyg.)
22. Halawani, A., Abdullah, A., and Saif, M.: Evaluation of the Efficiency of Miracil D in the Treatment of Bilharziasis in Egypt, Ztschr. Tropenmed. u. Parasitol. **8**:134-140, 1957.
23. Hawking, F.: Chemotherapy of Filarial Infections, Pharmacol. Rev. **7**:279-299, 1955.
24. Hill, A. W., and Andrews, J.: Relation of Hookworm Burden to Physical Status in Georgia, Am. J. Trop. Med. **22**:499-506, 1942.
25. Hirte, W.: Bandwurmkuren mit einem Zinnpräparat, Deutsche. med. Wchnschr. **76**:1083-1085, 1951.
26. Hoekenga, M. T.: Experiments in the Therapy of human Trichuriasis and Hookworm Disease, Am. J. Trop. Med. **5**:529-533, 1956.

26a. Jackson, F. C.: The Treatment of Tapeworm Infestation With Dichlorophen, South African M. J. **30**:853-854, 1956.

27. Jenkins, M. Q., and Beach, M. W.: Intestinal Obstruction Due to Ascariasis; Report of Thirty-one Cases, Pediatrics **13**:419-425, 1954.
28. Kuhls, R.: Zinn in der Bandwurmtherapie, Med. Klin. **48**:1511-1514, 1953.
29. Lecuona, M. de O.: Primeiros ensaios sobre a existência da teníase na Guiné Portuguesa e ensaio terapêutico com a Camoquina, An. Inst. trop. med., Lisbon **13**:87-96, 1956.
30. Magath, T. B., and Brown, P. W.: Standardized Method of Treating Tapeworm Infestations in Man to Recover the Head, J.A.M.A. **88**:1548-1549, 1927.
31. Mansour, T. E., and Bueding, E.: Action of Antimonials on Glycolytic Enzymes of Schistosoma mansoni, Brit. J. Pharmacol. **9**:459-462, 1954.
32. Mazzotti, L., and Mendez, D.: El Difentano 70 en el tratamiento de lag teniasis humanas, Rev. Inst. salub. y enferm. trop. **16**:9-14, 1956.
33. McCowen, M. C., Callender, M. E., and Brandt, M. C.: The Anthelmintic Effect of Dithiazanine in Experimental Animals, Am. J. Trop. Med. **6**:894-897, 1957.
34. Most, H.: Treatment of Schistosomiasis, Am. J. Trop. Med. **4**:455-459, 1955.
35. Mueller, J. F.: Studies on the Microscopical Anatomy and Physiology of Ascaris lumbricoides and A. megalocephala, Ztschr. f. Zellforsch. und mikr. Anat. **8**:362-400, 1929.
36. Miura, K.: Studies on Chemotherapy Against Hookworm Disease; on the Effect of 1-bromo-β-naphthol. Jap. J. M. Sc. & Biol. **7**:265-273, 1954.
37. Norton, S., and De Beer, E. J.: Investigations on the Action of Piperazine on Ascaris lumbricoides, Am. J. Trop. Med. **6**:898-905, 1957.
38. Otto, G. F., and Maren, T. H.: Use of Arsenicals in Filariasis, Ann. New York Acad. Sc. **50**:39-50, 1948.
39. Pena-Chavarria, A., Lizano, C., and Xirinachs, H.: The Treatment of Ascariasis With Piperazine Citrate in Typhoid Fever Patients, Am. J. Trop. Med. **6**:388, 1957.
40. Peters, L., Bueding, E., Valk, A., Jr., Higashi, A., and Welch, A. D.: The Antifilarial Activity of Cyanine Dyes (I), J. Pharmacol. **95**:212-239, 1949.
41. Robbins, B. H.: A Proteolytic Enzyme in Ficin, the Anthelmintic Principle of Leche de Higueron, J. Biol. Chem. **80**:251-257, 1930.
42. Rogers, E. W.: Excretion of Bephenium Salts in Urine of Human Volunteers, Brit. M. J. **2**:1576-1577, 1958.
43. Rosen, E.: Cortisone Treatment of Trichinosis, Am. J. M. Sc. **223**:16-19, 1952.
44. Sheth, U. K., Kekre, M. S., and Lewis, R. A.: Treatment of Ancylostomiasis With Win 5047, Antibiotic Med. **3**:197-198, 1956.
45. Shrapnel, B. C.: Oral Emetine in the Treatment of Intestinal Amebiasis, Am. J. Trop. Med. **27**:527-544, 1947.
46. Sodeman, W. A., and Jung, R. C.: Treatment of Teniasis With Quinacrine Hydrochloride, J.A.M.A. **148**:285-286, 1952.
47. Standen, O. D.: The Progress of Degenerat. Changes in Schistosomes Following the Treatment of Experimental Infections With 1:7 bis (p-dimethylaminophenoxy) heptane, Tr. Roy. Soc. Trop. Med. & Hyg. **49**:416-423, 1955.
48. Stoll, N. R.: This Wormy World, J. Parasitol. **33**:1-18, 1947.
49. Stoner, R. D., and Godwin, J. T.: The Effects of ACTH and Cortisone Upon Susceptibility to Trichinosis in Mice, Am. J. Path. **29**:943-950, 1953.
50. Swartzwelder, C., Miller, J. H., Frye, W. W., and Lampert, R.: Evaluation of the Efficacy of Piperazine Adipate for the Treatment of Patients With Trichuriasis, J. Parasitol. **42**: Sect. 2, 20, 1955.
51. Swartzwelder, J. C., Muhleisen, J. P., Abadie, S. H., Frye, W. W., Jones, C. A., Robertson, P. E., and Jones, C. A.: Therapy of Strongyloidiasis With Dithiazanine, Arch. Int. Med. **101**:658-661, 1958.
52. Swartzwelder, J. C., Frye, W. W., Muhleisen, J. P., Miller H., Lampert, R., Pena-Chavarria, A., Abadie, S. H., Anthony, S. O., and Sappenfield, R. W.: Dithiazanine, an Effective Broad-Spectrum Anthelmintic, J.A.M.A. **165**:2063-2067, 1957.

53. van Grunderbeeck, R., and Penson, D.: La taeniase en Ituri. Recherche d'une méthode de déparasitation massive adaptée a l'Ituri. Effets taenifuges de la Camoquin, Ann. Soc. belge méd. trop. **34**:981-998, 1954.

54. von Brand, T., Holtz, F., and Vogel, H.: Experimentelle Verkalkung unter dem Einfluss des Calcinosefaktors bei befall mit tierischen Parasiten, Ztschr. f. Parasitenk. **6**:308-322, 1933.

Sir William Osler, in his still arresting contribution to the Oxford Loose-Leaf Medicine, "The Treatment of Disease," states that: "In the progress of knowledge each generation has a double labour—to escape from the intellectual thralls of the one from which it has emerged and to forge anew its own fetters." For this occasion I would paraphrase Osler's sarcastic and rather gloomy statement as follows: Every practitioner has to fight a battle on two fronts; on the one hand against the erroneous views of preceding generations, though retaining what has proved to be right and, on the other, against the ever increasing flow of new therapeutic methods, in an attempt to separate the grain from the abundant chaff. Our main weapon in this fight is a critical attitude based on knowledge of the subject.

Our enemies on the first front are Authority, Tradition and Routine. Today we shall not dwell on this aspect of the battle; suffice it to say that defeat can only be avoided by examining ourselves every day as to the usefulness of what we are doing.

FROM "CRITICAL EVALUATION OF THE EFFICACY OF NEW DRUGS"
BY J. H. PANNEKOEK, POSTGRADUATE MEDICAL JOURNAL, VOL. 33, 1957.

Modern treatment of the systemic fungus diseases

For many years the systemic fungus diseases received little attention from the members of the medical profession and in the curriculum of the medical schools in the United States. Recently there has been a gradual awakening to their importance. The introduction of increasing numbers of antifungal chemotherapeutic agents attest this new interest in the mycotic diseases. Beneficial or curative specific therapy is now possible for many of these diseases which previously were without effective means of treatment.

E. Richard Harrell, M.D., and Florante C. Bocobo, M.D. *Ann Arbor, Mich.*

Department of Dermatology, University of Michigan Medical Center

The systemic fungus diseases are not common, and it is difficult to amass a significant number of patients with any one specific deep mycosis in order to evaluate clinically any new chemotherapeutic agent. This review has attempted to assemble and summarize the present-day knowledge of the newer antifungal agents. Individual consideration is given to the systemic fungus diseases: coccidioidomycosis, histoplasmosis, North American blastomycosis, South American blastomycosis, cryptococcosis, actinomycosis, nocardiosis, sporotrichosis, candidiasis, aspergillosis, and mucormycosis. Other contaminant fungi, such as species of Penicillium and Geotrichum, which at times assume a pathogenic role and cause disease, are not given individual consideration in this paper.

Since amphotericin B has given the most encouraging results of all the antifungal agents, greater attention is devoted to this antibiotic in this review.

Amphotericin B

Amphotericin B* is a polyene, antifungal antibiotic produced by a strain (M4574) of *Streptomyces nodosus* which was found in a soil sample obtained at Tembladora near the Orinoco River in Venezuela. It was first isolated and described by Gold, Stout, Pagano and Donovick[1] in 1955 and further characterized by others.[2-6]

Extraction of the whole culture broth in which the streptomycete is grown, or of the mycelial mat itself, yields a yellowish crude product containing amphotericin B, together with another active principle labeled amphotericin A. The extracting solvents used are water-saturated butanol; any of the lower alcohols, such as methanol, propanol, and, perferably, isopropanol; a water miscible solvent, like N,N-dimethylformamide or dimethyl sulfoxide. The two active principles may be separated from each other and purified by differential solution and precipitation. Further differentiation may

*Fungizone, E. R. Squibb & Sons.

be accomplished by specific optical rotation and ultraviolet absorption curves.

Physical and chemical properties.[1,4-7] Amphotericin B is a yellowish powder which is relatively insoluble. It is insoluble in anhydrous alcohols, esters, ethers, chloroform, benzene, toluene, ethyl acetate, glacial acetic acid, pyridine, or alcoholic potassium hydroxide. It is soluble only to a small extent in water at pH 2 and pH 11. Solubilized preparations, however, in the form of colloidal dispersions suitable for biologic work, have been made possible with the use of sodium desoxycholate. A melting point cannot be determined as the compound darkens and decomposes gradually above 170°C. The specific optical rotation in 0.1 methyl hydroxide-hydrochloric acid $[\alpha]_D^{23.5C}$ is −33.6 and in acid dimethylformamide $[\alpha]_D^{23.5C}$, +333. Its ultraviolet absorption curve is characteristic of a conjugated heptaene, like ascosin or trichomycin. The curve of amphotericin A is that of a conjugated tetraene, like rimocidin or nystatin.

Amphotericin B is amphoteric. The neutralization equivalent when it is titrated as a base is 929. It gives a negative ferric chloride test and a positive Molisch test and decolorizes potassium permanganate or bromide-carbon tetrachloride. Elementary analysis shows carbon, hydrogen, and nitrogen, but not halogen, sulfur, methoxyl or acetyl groups. The empirical formula of $C_{46}H_{73}O_{20}$ has been tentatively assigned to it. Evidence indicates a basic moiety of aminodesoxyhexose, for which the name "mycosamine" has been proposed.

Assay methods.[1,6,8-11] With the increasing use of amphotericin B, bioassay methods to determine the amounts of the drug in biologic fluids have been described, utilizing essentially the tube serial dilution technique or the agar diffusion method. These methods have not been standardized as yet. The test organisms used are *Candida albicans*, *Histoplasma capsulatum*, and *Saccharomyces cerevisiae*. Growth inhibition is measured by visual turbidity readings, turbidimetric determinations with the spectrophotometer, or direct counts of the organisms in a hemocytometer chamber.

Absorption, distribution, and excretion. Amphotericin B is poorly absorbed through the gastrointestinal tract in mice,[12,13] in dogs,[14] and in man.[6,8,12] Only maximal values of 0.03 to 0.08 μg per milliliter plasma were obtained in dogs 18 hours after a single oral dose of 500 mg. of amphotericin B. None was detected in the heart, liver, or spleen. Only 0.1 per cent of the daily dose was excreted in the urine during a 5 hour period, and 65 to 87 per cent was recovered from the stools during one 24 hour period.[14] In 13 patients given oral amphotericin B, the blood levels found were low. The spinal fluid frequently showed none, or very small amounts, of the drug. Only small quantities appeared in the urine.[8]

Clinical experience has shown that in patients who did not respond to the oral, subcutaneous, or even intramuscular administration of amphotericin B, assayable serum levels of the drug with resultant favorable clinical response could be achieved by intravenous infusion.[6,8,10,15] The blood level dropped considerably during the first 24 hours after intravenous administration, then gradually thereafter. It was still possible to demonstrate serum levels a month later.[11] The spinal fluid level is low, being 1/30 to 1/50 of the serum levels. The excretion through the urine is small and slow.

Toxicity in animals.[3,16] In acute toxicity studies with intravenous solubilized amphotericin B, the estimated lowest lethal dose (LD_2) and mean lethal dose (LD_{50}) for mice were 1.8 and 2.3 mg. per kilogram of body weight and 4.0 and 4.3 mg. per kilogram of body weight, respectively. In chronic toxicity studies, no toxic evidence was observed in two groups of 50 mice each given amphotericin B intraperitoneally in daily doses of from 5 to 100 mg. per kilogram of body weight for 50 and 71 days, respectively.

Apparently there are species differences. In rabbits, 62 per cent of 19 animals died 10 to 30 minutes after intravenous administration of 5.0 to 6.6 mg. per kilogram of

body weight. Death was preceded by a rise in the blood urea nitrogen and by tremors and convulsions. In dogs, intravenous doses as low as 1.0 mg. per kilogram of body weight elicited an unusual and specific intestinal reaction, with anorexia, emesis, hematemesis, and bloody diarrhea. Autopsy showed hemorrhagic involvement of the duodenum primarily. Three of 4 monkeys given 2.0 mg. per kilogram of body weight per day intravenously 5 days a week for 3 to 4 weeks showed neither acute nor delayed toxicity, except for occasional vomiting, sclerosis of veins, and elevation of the blood urea nitrogen.[7]

Toxicity in man. Oral amphotericin B is relatively nontoxic and, except for occasional mild gastrointestinal upsets, it is well tolerated, even in relatively large doses. When given intravenously the drug is more toxic, with a maximal tolerated dose of 1.0 to 1.6 mg. per kilogram of body weight. The chief toxic effects encountered are fever, nausea, emesis, anorexia, and azotemia, with the appearance of cellular and granular casts in the urine, as well as hematuria and proteinuria. The renal toxicity produced by amphotericin B represents the most serious drawback to the use of this antibiotic. The rise in the blood urea nitrogen which may occur during intravenous therapy is temporary and, after 2 to 7 days' rest, normal values are obtained. The antibiotic therapy can then be safely continued. The other toxic manifestations can be partially controlled by the use of chlorpromazine in a dosage of 50 mg. administered prior to the amphotericin B therapy, and soluble hydrocortisone in a dosage of 20 mg. administered with the intravenous amphotericin B suspension. There is question as to the wisdom of administering steroid hormones to patients with granulomatous disease; however, there has not yet been evidence of undesirable effect. The intravenous solution of amphotericin B should be administered by slow drip, and adjusted so that a minimum of 6 hours is required for the full daily dose. Toxicity can be minimized by strict attention to this simple procedure. The drug is best tolerated if administered every other day, rather than on consecutive days.

Antifungal spectrum. The results of the early in vitro studies[1-3,12,13,17] indicating marked fungicidal action of amphotericin B against a wide variety of pathogenic fungi, especially the systemic species *Candida albicans, Cryptococcus neoformans, Blastomyces dermatitidis, Blastomyces brasiliensis, Sporotrichum schenckii,* and *Histoplasma capsulatum,* have been substantiated by favorable findings in laboratory animal infections,[3,12,13,18-20] and by an expanding clinical experience in the treatment of human infections due to these fungi. Amphotericin B has no significant antibacterial activity.

Dosage and administration. The most effective mode of administration is by intravenous infusion. The parenteral soluble preparation of the drug comes as a sterile, lyophilized powder in vials each containing 50 mg. of amphotericin B and 41 mg. of sodium desoxycholate with a sodium phosphate buffer. Inasmuch as saline solution causes precipitation of the antibiotic, the recommended solvent is 5 per cent dextrose in water. The optimum concentration is between 0.1 mg. and 0.5 mg. per milliliter solution.

The optimum adult dose is 50 mg. per day, although the drug should be initially given at a dose of 25 mg. per day and gradually increased, depending upon the reaction of the patient. If toxicity develops with daily administration, the drug may be given on alternate days, or even stopped temporarily and resumed at a lower dosage level. The treatment course usually runs from 20 to 60 days, but may be longer if the clinical response of the patient is not satisfactory within that period.

When especially indicated, amphotericin B may be administered orally (2 to 10 Gm. daily), intrathecally (up to 1 mg. every other day), intramuscularly, intra-articularly (up to 25 mg. every other day), as an aerosol intrapulmonary spray (5 mg. every 6 hours), intrathoracically (up to 3 mg.), or

into the cutaneous lesions (up to 25 mg. with 2 per cent procaine every other day).[21]

Coccidioidomycosis

Coccidioidomycosis is one of the most prevalent and important of the deep mycoses. It is caused by *Coccidioides immitis*, a fungus indigenous to the arid regions of the southwestern United States. The principal focus of coccidioidomycosis is localized to the San Joaquin Valley of California, with known endemic areas in Nevada, Arizona, New Mexico, Texas, and northern Mexico.[22] Although the disease is acquired only in these areas, it has become a problem to all physicians regardless of their geographic location, for travel ultimately takes a large percentage of the United States population into the endemic areas. The arthrospore phase of *Coccidioides immitis* has been isolated from soil specimens, especially those taken from the burrows of desert rodents. These animals may serve as the reservoir for this disease.[23,24] Once infection develops in the human, the arthrospore, or vegetative phase of *Coccidioides immitis*, changes to a spherule stage. The spherule, which is laden with endospores, is seen on microscopic examination of infected human tissue. The organisms, which are quite characteristic in their shape, are normally 20 to 80 μ in diameter.[25] These spherules grow quite readily on artificial agar, producing a fluffy, white to brownish-white aerial hyphal growth, which segments into closely jointed structures called arthrospores. Such cultures are highly infectious and must be handled with great care by laboratory personnel.[26] There has been no known instance of human-to-human transmission of this disease.

Except for the rare instance of primary cutaneous inoculation coccidioidomycosis, the disease is acquired by inhalation of the causative arthrospore. When cutaneous inoculation occurs, a primary ulcerative granuloma resembling primary inoculation tuberculosis develops at the site of the inoculation. Satellite lymphadenopathy then develops, and the disease has been reported to run a completely benign course without evidence of dissemination.[27] Following inhalation of the causative agent by a previously uninfected person, the disease is usually confined to a focal area of the lungs, producing such mild disease that 70 per cent of the cases remain subclinical and unrecognized.[22] When symptoms occur, they are those of upper respiratory infection, usually revealing on roentgenograms of the chest areas of patchy infiltration, nodular infiltration, and pneumonitis, along with occasional pleural effusion and hilar lymphadenopathy. This type of patient may develop allergic manifestations such as erythema nodosum, erythema multiforme, or urticarial eruptions. Total healing takes place within a matter of several weeks to several months. Approximately 5 per cent of patients who have severe primary pulmonary coccidioidomycosis will be left with residual manifestations, consisting of thin-walled pulmonary cavitation. A much smaller percentage, less than 0.5 per cent of white-skinned persons, will develop disseminated coccidioidomycosis following primary pulmonary inhalation.[22] The Negro and Filipino are much more susceptible to the development of disseminated coccidioidomycosis than is the Caucasian.[28] When dissemination occurs, the spherules are spread via the blood stream and lymphatics throughout the body. There is no organ system which is immune to the development of coccidioidomycosis.

Treatment. No discussion of the therapy of coccidioidomycosis would be complete without mention of the immunology of the disease. Smith[29,30] has demonstrated very conclusively that a rising complement fixing antibody titer very closely parallels dissemination of the organism. As the complement fixing antibody titer rises, it is probable that the coccidioidin skin test sensitivity will be lost.

Until amphotericin B became available in 1956, there was no effective chemotherapeutic means of treating disseminated coccidioidomycosis. Prior to that time, the aromatic diamidines had been given a more

than adequate trial.[31-34] They were not effective in producing the same clinical improvement found with their use in North American blastomycosis. It must be concluded that they have little or no value in the treatment of disseminated coccidioidomycosis. The antibiotic, nystatin,* shows in vitro inhibition of *Coccidioides immitis*.[35-37] This drug, when administered intravenously or intramuscularly, causes severe chills, fever, malaise, and emesis to such extent that it is no longer in use. The oral absorption of nystatin from the gastrointestinal tract is so poor that no clinical effect can be obtained by the administration of the drug via this route. The broad-spectrum antibiotics, oxytetracycline, chlortetracycline, tetracycline, penicillin, as well as the sulfonamides, have not shown any in vitro inhibitory effect on *Coccidioides immitis*.[38] The sex hormone, diethylstilbestrol, has been reported to show in vitro effect on *Coccidioides immitis;* however, no clinical response has been obtained from such therapy.[39] Consistently good results have also been lacking with testosterone, methyltestosterone, and testosterone propionate.[40] There was evidence that the antibiotic, protoanemonin, would be clinically effective; however, this has not been substantiated.[41] Sodium caprylate, ethyl vanillate, fradicin, thiolutin, and prodigiosin have been ineffective in the treatment of the systemic form of the disease.[42-44] Many other chemotherapeutic agents besides those mentioned have been tried in this systemic fungus disease. They have all been universally ineffective in controlling the spread of the causative agent, or preventing death from the disease.

Amphotericin B is the treatment of choice for coccidioidomycosis, when therapy is indicated. Indications for treatment include: (1) primary pulmonary coccidioidomycosis in the Negro or Filipino, (2) a residual pulmonary cavity, which is increasing in size and causing increasing hemoptysis, or which has ruptured with spontaneous pneumothorax or empyema, (3) rising complement fixing antibody titer well beyond the time of the primary pulmonary infection, (4) any clinical evidence of dissemination of the disease, (5) any type of surgical procedure, particularly corrective pulmonary surgery, (6) pregnancy, since this condition is associated with an increased risk of dissemination. The first case report of the use of amphotericin B in coccidioidomycosis was by Halde, Newcomer, Wright, and Sternberg.[12] This preliminary report recorded a failure from the use of the drug administered orally. The second report by Fiese[45] recorded a favorable response from large amounts of amphotericin B given orally. Klapper, Smith, and Conant[46] recorded an apparent cure with orally administered amphotericin B in amounts of 4 Gm. per day for 10 months, followed by 2 Gm. per day for an additional 4 months. Other authors[47,48] have reported failures following the use of amphotericin B given orally. It is apparent that the greatest effect from this drug is obtained when it is administered intravenously. Littman, Horowitz, and Swadey[6] have reported excellent results with the intravenous use of amphotericin B in 4 patients with disseminated coccidioidomycosis. Other authors[48,49] have also reported benefit from this antibiotic when given in adequate amounts intravenously. It is difficult to understand why the 3 patients with disseminated coccidioidomycosis discussed by Utz, Treger, McCullough, and Emmons[50] failed to show any response following an adequate intravenous course of this drug. It is not likely that these 3 patients had strains of *Coccidioides immitis* which had become resistant to amphotericin B. Such resistance has been artifically developed in vitro.[51] We have had the occasion to treat one patient with coccidioidomycosis with intravenous amphotericin B. In April, 1957, a 28-year-old white male U.S. Army veteran was seen at the University Medical Center in Ann Arbor, Michigan, for an isolated cavity of the right upper lung. The patient related that he had been aware of the cavity for the 5 years following his discharge from the U.S. Armed Forces. He had been stationed in the San

*Mycostatin, E. R. Squibb & Sons.

Joaquin Valley during part of his time in the United States Army. One unsuccessful attempt at surgical removal of the cavity had been performed elsewhere before the patient was seen by us. The case was interpreted as one of a solitary, thin-walled cavity as the sole remaining evidence of previous primary pulmonary coccidioidomycosis. No evidence of dissemination was present. *Coccidioides immitis* was readily found on examination of the patient's sputum, and positive cultures were obtained on Sabouraud's glucose agar. The patient's chief complaint was that of increasing hemoptysis. He did not desire further surgery. He was treated with amphotericin B, administered in a daily dosage of 50 mg. dissolved in 1,000 c.c. of 5 per cent glucose in water given by slow intravenous drip on consecutive days for 20 days. He received a total dose of 1.0 Gm. of amphotericin B. By the time the patient had received the sixth injection of amphotericin B, his sputum became mycologically negative, and all evidence of hemoptysis ceased. He ceased to raise sputum altogether by the time the course of 1.0 Gm. of the drug had been given. He developed a mild febrile response and nausea with each intravenous injection. Prior to the institution of amphotericin B therapy, the cavity measured 3 cm. in diameter by x-ray examination. One year following the amphotericin B therapy, the patient had remained completely asymptomatic. There was still x-ray evidence of pulmonary cavity; however, it had decreased in size to approximately 1 cm. in diameter. He has since been lost from our observation. This patient had a negative coccidioidin skin test, and his serum was negative for complement fixing antibodies.

Although a very limited number of patients have been reported to be treated with amphotericin B via the intravenous route, there is good evidence that this rather toxic antibiotic has a profound in vivo antifungal effect. The optimum total dose of amphotericin B remains to be determined.

Surgical excision of an isolated pulmonary cavity should be considered if the cavity cannot be closed by amphotericin B therapy or by pneumothorax or pneumoperitoneum. Complications accompanying pulmonary operation for coccidioidal cavities have been few, but relatively serious. Any surgical procedure performed on a patient with coccidioidomycosis should be accompanied by amphotericin B therapy.

Histoplasmosis

Numerous epidemiologic studies and histoplasmin skin test surveys have radically changed the old concept of histoplasmosis as being a rare and fatal disease. It is now known on all continents, with the highest reported endemicity in the East Central states of the United States bordering the tributaries of the Ohio, Missouri, and Mississippi rivers.[52,53] In these endemic areas, the incidence of histoplasmin reactors may reach the figures of 80 to 90 per cent of the population.

The disease is caused by *Histoplasma capsulatum*, which is dimorphic like *Blastomyces dermatitidis*, *Blastomyces brasiliensis*, and *Sporotrichum schenckii*. The tissue forms are small, 1 to 5 μ in size, round or oval, yeastlike budding organisms, predominantly located in reticuloendothelial cells. The saprophytic phase is mycelial, producing characteristic tuberculate chlamydospores which are considered as the infective structure of the organism in natural exposures. The reservoir in nature is the soil, as indicated by repeated isolation of the organism from soil samples—particularly those associated with chicken and bat droppings from endemic areas and from point sources of reported epidemics.[54] Evidence points to histoplasmosis as being an airborne infection, with the respiratory tract as the major portal of entry. Under normal conditions of exposure the infection may be so mild as to be asymptomatic; or, if symptomatic, after an incubation period of 5 to 15 days, it may appear as an influenza-like or respiratory condition, usually mistaken for a common cold, tuberculosis, bronchopneumonia, or other nonmycotic

ailments. The x-ray findings are hilar adenopathy and/or parenchymal infiltrates. Precipitins and complement fixing antibodies appear, and the histoplasmin skin test becomes positive. As a rule, the patient recovers after a few weeks, although the pulmonary lesions may persist and eventually become calcified. Not more than 1 per cent of the primary infections progress to the disseminated, fatal form, although recently more and more cases of the chronic progressive pulmonary type of histoplasmosis (which closely resembles chronic fibrocaseous pulmonary tuberculosis) are being recognized, particularly in tuberculosis sanatoria.[55] The disseminated form with fever, weakness, anemia, wasting, and hepatosplenomegaly is the classical clinical picture by which histoplasmosis was previously known.

Histoplasmosis is generally regarded as a protean disease running the gamut of symptomatology from a mild, almost subclinical upper respiratory infection to an overwhelming, fatal septicemia, with the clinical picture related to the sites involved. Furcolow[56] described the following clinical types:

1. Severe
   a. chronic progressive (reinfection or cavitary)
   b. acute progressive
   c. acute epidemic
2. Moderately severe
3. Mild
4. Asymptomatic

The significance of immunologic phenomena in histoplasmosis parallels that in coccidioidomycosis and North American blastomycosis.[57] Precipitins and complement fixing antibodies become measurable during the early stage of the infection. They disappear as the illness subsides, thus indicating the severity and extent of the disease process. The skin hypersensitivity, as demonstrated by the histoplasmin skin test, develops from 4 to 6 weeks after the onset of the disease and persists for many years. Its significance is similar to that of the tuberculin test, indicating a past or present infection. It may be used as a measure of resistance of the body to the infection. The complement fixation test and the histoplasmin skin test are interpreted together for diagnostic and prognostic purposes and are valuable tools in the evaluation of the effectiveness of the treatment instituted.

Treatment. The extreme variation in the clinical picture and course of histoplasmosis makes the evaluation of therapeutic modalities difficult. Immunologic mechanisms apparently play a significant role and should be considered in the response of the patient to therapy, which varies markedly depending upon the severity and clinical type of the infection. This becomes important in the absence of an effective etiological treatment.

The acute primary pulmonary infection is generally benign and self-limited, thus nonspecific supportive and symptomatic measures suffice. Many patients do not even seek medical assistance. However, this type of case may occasionally become severe enough to threaten the life of the patient as may occur in the acute epidemic form with unduly massive exposure to the infective organism.[58] The question of the use of steroids in these cases has been raised in spite of their known association with dangerous spread of infectious processes. Although Baum, Adriano, and Schwarz[59] failed to observe any influence of cortisone on the dissemination of experimental histoplasmosis, others[60-62] obtained evidence of wider spread. In clinical trials, certain authors noted an association of the spread of the infection with administration of steroids,[63-65] while others thought that the steroids contributed to the clinical improvement and the clearing of lesions.[66-68] A special indication for the use of steroids in histoplasmosis has been pointed out by Schwartz[69] and Packard, Finkelstein, and Turner.[70] Carefully controlled administration of steroids may even prove lifesaving by virtue of their anti-inflammatory and antitoxemic effects in patients threatened by a self-limited but massive acute pulmonary infection with severe toxemia and high fever.

The drugs, however, should not be given during the first 2 weeks of illness when the body has not yet had the chance to build up adequate immunologic defense. The risk of dissemination may be minimized by the concomitant administration of one of the more successful histoplasmostatic agents as was done by Tegeris and Smith,[68] who used cortisone and MRD-112. The use of amphotericin B as an antifungal "cover" may be more promising.

The problems encountered in the management of chronic pulmonary histoplasmosis are similar to those in chronic pulmonary tuberculosis.[55] The indications for sanatorial treatment, supportive and symptomatic measures, and surgical intervention are similar, except for the lack of an accepted specific drug in histoplasmosis. Surgical excision of localized lesions has proved of definite value.[71-74]

The almost uniformly fatal course of progressive disseminated histoplasmosis highlights the need for an effective specific drug. The newer drugs that have shown a certain measure of success are ethyl vanillate, β-diethylaminoethyl fencholate (MRD-112), and sulfadiazine, but they are being discarded in favor of the more effective amphotericin B. The aromatic diamidines and nystatin are generally considered ineffectual in clinical trials.

Ethyl vanillate (ethyl-4-hydroxy-3-methoxybenzoate) was first used clinically in 1951 by Christie, Middleton, Peterson, and McVickar[75] on 12 children with disseminated histoplasmosis. Five recovered. Subsequent trials by other authors[76-84] gave variable results. The drug is administered orally in large doses up to 40 Gm. It causes distressing gastrointestinal irritation and serious toxic reactions, with a narrow margin of safety between the toxic and the therapeutic doses. These are distinct disadvantages, especially with the introduction of other drugs, equally effective but less toxic.

Ludwig, Murray, Smith, Thompson, and Werner[85] demonstrated the in vitro antifungal activity of MRD-112 at a concentration of 0.1 mg. per milliliter of medium. As with ethyl vanillate, the therapeutic results in humans with the use of MRD-112 have been inconsistent.[66,84,86-91] The drug is given for 1 to 2 months in daily doses of 150 to 600 mg. as a slow intravenous drip. Except for some evidence of hepatic degeneration, it has not shown serious toxicity.

The results of laboratory studies on the in vitro and in vivo antifungal activity of the sulfonamides on *Histoplasma capsulatum* have been contradictory, although the recent reports of Mayer, Eisman, Geftic, Konopka, and Tanzola[92] and Louria and Feder[93] agreed on their effectiveness in protecting mice against experimental histoplasmosis. The clinical use of sulfonamides in human histoplasmosis has generally been regarded as disappointing. There are, however, reports of favorable results, and, recently, Christie[94] has reinvestigated the problem by treating 3 cases of progressive disseminated histoplasmosis and 2 cases of the ulceroglandular type with sulfonamides. All five achieved clinical remission. It is to be noted that Latin-American authors have taken exception to the general opinion that sulfonamides are ineffective in histoplasmosis. Their successful experience in improving the prognosis of South American blastomycosis with the use of sulfonamides has led to their use of these drugs in cases of histoplasmosis, resulting in more satisfactory clinical responses and a number of apparent clinical cures through the administration of large doses for prolonged periods.[95-101] Cordero[98] felt that most of the reported clinical failures were due to relatively small doses or short periods of administration. As in South American blastomycosis, the sulfonamide preparations, particularly sulfadiazine, are given orally in daily doses of 3 to 6 Gm. for months.

The best therapeutic results so far have been obtained with the use of amphotericin B. The number of cases reported is still relatively small, and the periods of post-treatment observation still too short for exact evaluation of its value; but the preliminary findings are certainly promising.

The inhibitory in vitro concentration of the drug against *Histoplasma capsulatum* is very low, as little as 0.04 μg per milliliter of medium.[1,3,13,102] The drug is highly protective in experimental histoplasmosis in animals.[2,19,20,103] Amphotericin B has been tried on at least 34 cases of human histoplasmosis.[11,16,20,50,91,104-110] Two patients with acute pulmonary histoplasmosis improved. Although the fall in temperature and the rapid resolution of the infiltrates coincided with the institution of therapy with amphotericin B, the claim of improvement is at most suggestive in view of the good prognosis of this form of histoplasmosis. Nine cases of the chronic pulmonary type were treated. The symptoms were arrested in one, improved in 7, and no change appeared in one case with oral administration of 2.0 to 4.0 Gm. of the drug daily for 40 days. Eighteen cases of progressive disseminated histoplasmosis were treated. Seven clinical remissions were observed, with follow-up periods ranging up to 18 months. Nine patients improved dramatically. One also showed improvement with oral treatment, but died in 7 months. Three other deaths occurred, but the drug was administered only a few times during the terminal stages of the disease. Certainly these figures are unparalleled.

North American blastomycosis

North American blostomycosis is caused by the fungus, *Blastomyces dermatitidis*, an organism which grows readily on Sabouraud's glucose agar at room temperature as a filamentous mold, and on blood agar at 37° C. as a yeastlike fungus. The tissue phase of the fungus appears as a characteristic single-budding structure of 5 to 15 μ in diameter, and exhibits a refractile cell wall from which the protoplasm has often shrunk away, giving a double-contoured appearance. The polysaccharide wall of the organism is well demonstrated in tissue by means of the periodic acid–Schiff stain. The diagnosis of North American blastomycosis can be established by cultures or by the finding of the causative agent on direct microscopic examination of pus obtained from the cutaneous lesions or from other infected clinical material.

North American blastomycosis is most frequently encountered in the Southern, Southeastern, and Midwestern areas of the United States. Cases of the disease have been reported sporadically from other geographic areas of the United States as well as Canada.[111] There is doubt that the disease has been acquired outside of the North American continent, even though case reports from other continents are in the medical literature. *Blastomyces dermatitidis* has not, as yet, been isolated from the soil in which it must logically exist.[112] The organism shows a decided tendency to produce disease in the male sex. Reported ratios of men to women average approximately ten to one.[113-115] The disease has now been reported in siblings[112] and develops in all age groups. One small epidemic of North American blastomycosis has recently been reported.[112]

North American blastomycosis is nearly always acquired by inhalation of the causative fungus. This somewhat revolutionary thought was first advanced by Schwarz and Baum,[116] and it has been slow to be generally accepted. The rare person who has had known primary cutaneous inoculation blastomycosis has followed a clinical pattern resembling that of primary inoculation tuberculosis or sporotrichosis.[117,118] There has been no instance recorded of known primary inoculation blastomycosis developing into a systemic blastomycosis. There is now evidence that a subclinical form of pulmonary blastomycosis occurs, similar to that encountered in coccidioidomycosis and histoplasmosis.[112,113,115] The blastomycetes are disseminated from the lungs via the blood stream and lymphatics to other organs of the body, with a predilection for the skin, subcutaneous tissue, and bone. Pulmonary blastomycosis mimics most of the other deep mycoses, as well as tuberculosis, while the cutaneous granulomas also closely simulate tuberculosis, tertiary syphilis, other deep mycoses, and certain drug eruptions.

Treatment. The therapy of North American blastomycosis should not be discussed without emphasis, first, upon the importance of correlating the results of therapy with the immunologic status of the patient. Smith[119] was the first to recognize that the prognosis of a patient with blastomycosis is dependent to a large extent upon the immunologic response of the individual. The prognosis is best in patients who have a positive blastomycin skin test and no demonstrable complement fixing serum antibodies. Spontaneous cure has been reported[112] in a patient who exhibited this immunologic pattern. The prognosis is poorest in those patients who have a negative blastomycin skin test and a high titer of complement fixing serum antibodies.

2-Hydroxystilbamidine and amphotericin B, both administered intravenously, represent the two present choices for chemotherapy of North American blastomycosis. Evidence is accumulating that amphotericin B will prove to be superior to 2-hydroxystilbamidine. The use of 2-hydroxystilbamidine in generalized North American blastomycosis was first reported by Snapper, Schneid, McVay, and Lieben.[120] This aromatic diamidine does not cause the toxic neurological effect which was almost universally recorded with stilbamidine when the latter drug was given in doses greater than 1 Gm.[120-125] Very excellent reviews have been reported on the aromatic diamidines.[120,126,127] 2-Hydroxystilbamidine has gained widespread acceptance in the therapy of North American blastomycosis.[38,115,128] This drug is supplied in vials of 225 mg. The dose of 450 mg. of the drug is as well tolerated as is the smaller 225 mg. dose. This amount of 2-hydroxystilbamidine is dissolved in 500 to 1,000 c.c. of 5 per cent dextrose solution or isotonic saline and administered intravenously by slow drip. The solution is made up each day, just prior to its administration. Care should be taken to keep the drug from direct sunlight, since it has been well documented that toxicity of the compound increases upon exposure to direct sunlight. The drug is administered on consecutive days, although rest periods of days to weeks may safely be given. A course of 9.0 Gm. of 2-hydroxystilbamidine represents an adequate course of the drug. Healing of the cutaneous lesions and symptomatic improvement are usually noted by the end of the second week of therapy. Complete healing has usually taken place by the end of the first month following the cessation of therapy. 2-Hydroxystilbamidine is deposited in various organs, especially the liver, kidneys, adrenals, sweat glands, and skin,[120,127] and continued improvement occurs for at least several weeks following the last administration of the drug.

There are reports documenting relapse of North American blastomycosis following adequate amounts of aromatic diamidine therapy.[129-131] Most of these recurrences have been in individuals who have contributed little to their own immunologic defense, as evidenced by negative blastomycin skin tests and positive complement fixing antibody tests. There is also evidence that resistance to 2-hydroxystilbamidine is developed by specific strains of *Blastomyces dermatitidis*.[132]

Intravenous amphotericin B therapy has proved effective in 8 cases of North American blastomycosis treated at the University of Michigan Medical Center.[118] Five of these successes occurred in patients who had exhibited repeated relapses following massive amounts of aromatic diamidine therapy.[118] Utz, Treger, McCullough, and Emmons[50] have reported 4 cases of systemic North American blastomycosis treated with intravenous amphotericin B. One of these 4 patients received only 110 mg. total amount of the drug. This does not represent an adequate amount of therapy. The other 3 received between 2.2 and 5.1 Gm. of amphotericin B in daily amounts varying between 30 and 45 mg. per dose. This averaged between 0.5 and 0.6 mg. per kilogram of body weight. These 3 patients are reported to have made apparent recovery, although an adequate follow-up time has not yet elapsed to evaluate these results thoroughly. Toxic symptoms in the form of

nausea, vomiting, marked anorexia, chills, and fever were reported in the same patients. Nevertheless, these authors conclude that amphotericin B is the drug of choice for disseminated North American blastomycosis.

Five of the 8 patients reported upon by us received but 1.0 Gm. of amphotericin B as a total dose. All 5 of these patients have apparently been cured, with a follow-up time varying from 11 to 29 months. Three of our 8 patients have had a relapse following amphotericin B therapy. One of these 3 was the first patient we treated with this antibiotic, and in retrospect we realize that he had received an inadequate amount of the drug when first treated. He was then given 1.0 Gm. of the drug, bringing his total dose to 1.5 Gm. He is apparently cured at the present time, being 26 months post treatment. The 2 other patients who suffered a relapse have received 4.0 and 4.5 Gm. of amphotericin B, respectively. These 2 patients had had widespread disease for many years prior to amphotericin B therapy. Each course of retreatment with this antibiotic has proved to be effective in producing temporary cessation of all signs and symptoms of active disease. These two patients are currently 14 and 15 months post treatment without evidence of active infection. It appears to us that a smaller total dosage of amphotericin B is required in the treatment of North American blastomycosis than is required in the therapy of any of the other systemic mycoses. The dosage of 50 mg. of amphotericin B given by slow intravenous drip, with the concomitant use of judicious amounts of steroid hormones and chlorpromazine at intervals of every other day, to a total dose of 1.0 Gm., represents the most effective means at hand of curing North American blastomycosis.

The oral form of amphotericin B is poorly absorbed, and, in spite of the one case[133] reporting benefit from such oral administration, we do not feel that this form of amphotericin B therapy should be given further trial.

Prior to the use of the aromatic diamidine and amphotericin B, a number of drugs had been tried for this disease. None of them have proved consistently effective. A saturated solution of potassium iodide administered orally and in increasing amounts had proved the only form of therapy which promised any beneficial effect. The cures which have been reported from the use of iodides usually occurred in individuals with the immunologic status of a positive blastomycin skin test and no complement fixing serum antibodies. More recently, Cornbleet[134] has advocated the use of a solution of potassium iodide plus thyroid extract in the treatment of North American blastomycosis. The 9 patients included in his report responded impressively to this form of therapy. There have been no follow-up or corroborative studies reported. There is doubt that there is any remaining place for iodide therapy or any of the older means of therapy advocated prior to the introduction of 2-hydroxystilbamidine.

South American blastomycosis

South American blastomycosis has been reported almost exclusively from South America, especially from Brazil. With increased opportunities for travel and greater population movements, however, cases may be encountered in countries distant from the known endemic areas, as exemplified by occasional reports from Germany,[135] Italy,[136] and the United States.[137,138]

The causative organism, *Blastomyces brasiliensis*, is a dimorphic fungus with filamentous growth at room temperature and yeastlike morphology at 37° C. The organism is characterized by multiple budding. Even though it has not been isolated yet from soil or plants, evidence points to a saprophytic phase in nature. The portal of entry is mainly the mucous membranes of the mouth, pharynx, or respiratory tract, accounting for the preponderant occurrence of the granulomatous lesions in these areas. From these locations, the infection disseminates through the blood stream and the lymphatic system to affect any organ, caus-

ing grave septicemic lesions. The lymph nodes and gastrointestinal tract are commonly involved. Lacaz[139] described the following anatomoclinical forms: (1) mucocutaneous, (2) ganglionary (lymphatic), (3) visceral, (4) mixed, and (5) blastomycosis associated with neoplasms and other infectious diseases.

Experience with the paracoccidioidin skin test and complement fixation test in South American blastomycosis is as yet limited and the results inconsistent.[57] Better standardization and more studies may yet prove these procedures as valuable in the diagnosis and prognostication of South American blastomycosis as of coccidioidomycosis, histoplasmosis, and North American blastomycosis.

Treatment. The prognosis of South American blastomycosis is generally grave. In some cases death may occur in 6 months, but the usual patient suffers through 2 to 4 years. The use of iodides, arsenicals, bismuth, gold, antimony, and certain dyes, like malachite green and methylene blue, had not modified significantly the serious course of the disease. However, since Ribeiro first treated a group of patients with sulfonamides in 1940 and obtained favorable results, the prognosis in this disease has been greatly improved. The response to sulfonamide therapy is satisfactory and the life expectancy of the patient is increased. Even biologic cures, though relatively few, have been claimed. To attain these, the administration of the drug must be early, intensive, and prolonged. Any of the sulfonamide preparations may be used singly or in combination. Sulfamerazine, Gantrisin, and, especially, sulfadiazine are preferred. The daily oral dose is 1 to 2 Gm. every 4 to 6 hours. In this way the effective blood level of 10 to 15 mg. per 100 ml. is attained and must be maintained continuously for long periods of time, even after apparent clinical cure. Relapses are common, particularly when administration of the drug is suspended. The recurrences are resistant to the resumption of therapy. The protracted administration of such large doses of sulfonamides that the total dose may reach even a few thousand grams entails the risk of toxicity. Periodic examinations for such toxic effects should be made.

In spite of the notable improvements attained with sulfonamides, the relapses, the toxicity of or intolerance to the drugs, the few actual mycologic cures, and the protracted therapy comprise the incentive to search for better agents. The antibacterial antibiotics are ineffective, as are ethyl vanillate[140] and nystatin.[141] Floch and Saccharin[142] claimed a clinical cure with 4,4′-diaminodiphenyl sulfone (DDS).

The hope that the favorable therapeutic effect of stilbamidine or 2-hydroxystilbamidine on North American blastomycosis would be of the same magnitude, if not greater, on South American blastomycosis has not been fulfilled. *Blastomyces brasiliensis* clinically has proved much less sensitive.[138,143,144] Another aromatic diamidine, diamidinodiphenylamine (M & B 938), proved to be more promising, however. MacKinnon, Sanjines, and Artagaveytia-Allende[144] tested six strains of *Blastomyces brasiliensis* and found the inhibitory concentration at 2 to 5 mcg. per milliliter of medium. They observed healing of oropharyngeal lesions and marked improvement of lung changes in a patient given two courses of daily intravenous injections of 150 mg. of M & B 938 with a total of 4 Gm. Another patient also showed definite improvement with a total of 5.475 Gm. in 43 days. There was no serious toxic reaction.[145]

In their initial studies of the in vitro antifungal spectrum of amphotericin B, Gold, Stout, Pagano, and Donovick and Sternberg, Wright, and Oura included *Blastomyces brasiliensis* which turned out to be sensitive. On the strength of these findings, Lacaz and Sampaio[146] treated 4 cases of South American blastomycosis with amphotericin B. The clinical results they obtained were so promising as to prompt them to state that a new era in the treatment of South American blastomycosis had opened with amphotericin B. Although kept alive by sulfonamide therapy, all 4 patients were essentially

refractive to the drug. One patient had taken more than 10,000 Gm. of sulfonamides in the course of 19 years, and another, 7,500 Gm. in 10 years. The relapses in each patient were controlled by amphotericin B with healing of the lesions, but no cure could be claimed, for the follow-up periods were still too brief.

Cryptococcosis

The true incidence of cryptococcosis is probably much higher than is indicated by the case reports in the literature of the severe pulmonary and meningoencephalitic forms with which most clinicians associate the disease. In the absence of a high index of suspicion and effective skin testing and serologic procedures, many of the benign, localized forms of cryptococcosis that simulate more commonly known diseases may go on to healing without being diagnosed. The condition has been reported from many countries and in both sexes and all ages.

The culturally and morphologically yeastlike organism, *Cryptococcus neoformans*, causes the disease. It is unique among the pathogenic fungi in the mucoid character of its colonies and the wide capsule surrounding its yeastlike body. The organism has been isolated from the skin and gastrointestinal tract of normal individuals, but more frequently from soil samples, especially those associated with pigeon and chicken droppings.[147,148] The infection may be endogenous, but probably more commonly results from an exogenous source through the respiratory tract and, occasionally, through the skin or gastrointestinal tract.

Because the vast majority of reported cases of cryptococcosis showed marked involvement of the central nervous system, the disease has become popularly associated with this particular form. In accord with the suspected mode of entrance of the organism through the respiratory tract, however, more and more attention has been directed to the pulmonary form of the disease. In many cases pulmonary complaints were registered at one time or another during the course of the illness. Although primary pulmonary cryptococcosis may at times cause death in a short time, the infection generally runs a benign, even subclinical, course with localized lesions that may resolve with or without minimal scarring. Eight of the 10 cases of pulmonary cryptococcosis described by Jacobs[149] revealed a solitary granulomatous nodule. These primary lesions in the lungs, whether active or healed, are frequently discovered only at autopsy on patients who die of the disseminated or meningitic form. The signs and symptoms of pulmonary cryptococcosis are those of chronic pneumonitis, lung tumor, or lung abscess.

With no regard to the course taken by the primary pulmonary condition, the infection may be disseminated hematogenously to other organs, with a predilection for the central nervous system. When this happens, the clinical picture becomes dominated by the central nervous system involvement, with signs and symptoms of meningeal irritation or increased intracranial pressure frequently misdiagnosed as tuberculous meningitis, brain tumor, brain abscess, or subarachnoidal hemorrhage. Involution of the pulmonary lesions may continue even in the face of this dissemination. Cryptococcosis of the central nervous system is almost always progressive, generally causing death in 3 to 4 months, sometimes in one year or so. However, unexplained remissions do occur and the patient may live up to 16 years, as in Beeson's[150] case.

Treatment. There is as yet no generally accepted effective treatment of disseminated cryptococcosis. Evans and Harrell[151] found that at least 43 methods, including the usual antibiotics, sulfonamides, x-rays, fever therapy, alkalinization, gold, copper, arsenicals, iodides, and vaccines, had been used. All proved essentially ineffectual. Except for amphotericin B, even the newer antifungal antibiotics and chemotherapeutic agents effective in vitro and in other mycotic infections give variable results, which suggests that possibly the apparent cures claimed for these drugs were actually spon-

taneous remissions. The observed resistance of *Cryptococcus neoformans* to drug therapy has been related to the thick capsule surrounding the organism.

Because of their in vitro activity[152,153] and demonstrated usefulness in the treatment of North American blastomycosis, the aromatic diamidines have been tried on clinical cases of cryptococcosis, in spite of failures in protecting laboratory animals against experimental infections.[154-156] Leithold, Reeder, and Baker[157] and Whitehill and Rawson[158] claimed apparent cure with 2-hydroxystilbamidine in one case each, and Ferguson[159] achieved marked improvement in another. The use of stilbamidine or propamidine, however, has been associated with clinical failure.[159-163]

Nystatin has been found active in vitro[164,165] and in animal experiments.[35,156,166] However, unlike its signal success in candidiasis, the few trials in human cryptococcic infections have thus far essentially failed to confirm such activity.[167-171] Perruchio, Bruel, Lagarde, and Delpy[172] used sulfadiazine and nystatin to treat a residual pulmonary lesion after resection in a 12-year-old girl. The sulfadiazine had to be stopped because of digestive intolerance but nystatin was administered for 20 days without trouble. The pulmonary lesion remained stationary.

Cycloheximide (actidione) is an antifungal antibiotic that has a selective marked in vitro activity against *Cryptococcus neoformans* among the pathogenic fungi.[35,164,173] It is not active, however, in experimental infections.[174] Likewise, in clinical trials only an occasional patient responded favorably.[175-178] The majority were not influenced and, in one instance, the drug might have even brought on an acute exacerbation of the disease and caused death.[167,179-185] The antibiotic is given intravenously, intrathecally, and/or intramuscularly. The initial dose is 20 mg., increased daily up to 100 to 200 mg. The intrathecal dose is 10 to 20 mg. The treatment is prolonged. Except for nausea and vomiting that may be severe, no serious toxicity has been observed.

In contrast with the other drugs, amphotericin B has exhibited a most encouraging activity. So far it is the most effective drug in the treatment of cryptococcosis and is apparently changing the gloomy outlook in this grave disease. It inhibits the organism in vitro at very low concentrations, down to a fraction of a microgram per milliliter of medium.[1,13,102] The increasing clinical use has consistently produced favorable results hitherto unknown in the management of cryptococcosis. Thus far, at least 49 patients, the majority of whom had central nervous system involvement, have been reported treated with amphotericin B.[11,16,50,91,110,168,171,186-197] Apparent clinical remissions were claimed in 17 cases with follow-up periods of from 4 to 18 months. Nineteen improved in varying degrees, with 8 noted as having attained conversion of the cultures for the organism to negative. Three patients were reported unimproved, but in 2 of them the drug was administered mainly by the oral route. Two relapses and 8 deaths occurred, but in 6 cases medication was instituted late in the course of the disease a short time before death, and the authors expressed the opinion in each case that the treatment was not given a fair trial. In one case the organism disappeared from the spinal fluid, and death was blamed on bacteremia and renal failure.

Surgical intervention has a definite place in the management of cryptococcosis, being particularly indicated for excision of early, localized lesions before dissemination occurs.[198-202] The resection of primary lesions has been attempted even in the presence of spread to the central nervous system in the hope of preventing further seeding from these primary foci.

Actinomycosis

Actinomycosis is a subacute to chronic suppurative granulomatous disease characterized by brawny inflammatory lesions, abscess formation, and multiple draining sinuses and fistulas. It is world-wide in distribution with no racial predilection and affects all ages, especially young adults.

The single causative organism is *Actinomyces israeli* (syn. *Actinomyces bovis*) which is gram positive, non-acid fast, branching, filamentous, and anaerobic. The diagnostic "sulfur granules" found in the exudate from the abscesses or draining lesions are dense networks of the filamentous organism radially arranged, with clublike endings.

Actinomyces israeli has been isolated from the gums, periodontal pockets, and peritonsillar crypts of normal individuals. The infection is apparently endogenous, with trauma or disease as contributory factors related to lowered local tissue resistance and acquired pathogenicity of the organism. "Mixed infection" with other symbiotic bacteria, as *Actinobacillus actinomycetem-comitans, Bacterium melaninogenicum,* and anaerobic streptococci has been implicated as part of a pathogenic complex with *Actinomyces israeli.*[203-206]

A wide variety of signs and symptoms is observed in actinomycosis depending upon the part of the body invaded. The three main clinical types are (1) cervicofacial, comprising more than half of the reported cases, (2) abdominal, 20 per cent, and (3) thoracic, 15 per cent. The rest of the cases present involvement of other parts of the body such as the extremities, liver, genitals, kidneys, skin, bones, joints, or central nervous system. Spread of the infection is by direct invasion. The prognosis of cervicofacial actinomycosis is generally much better than that of the abdominal or thoracic types.

Treatment. Before the advent of sulfonamides and penicillin in the treatment of actinomycosis, a multitude of therapeutic measures (including iodides, x-rays, surgical procedures, vaccines, copper salts, thymol, gold, arsenicals, and others) were utilized with varying results. Singly, they were ineffective, especially in the abdominal and thoracic forms of the disease. Even in combination, most of the reports of their favorable use were on cases of cervicofacial actinomycosis which has a relatively fair prognosis, even without therapy. Many of these older methods have been discarded except for a few, such as iodides and x-rays, which are retained by some as helpful adjunctive measures to penicillin treatment.

Although later proved to be less effective than penicillin and the other antibiotics in vitro[207,208] and in clinical treatment,[209-211] the sulfonamides gave the first consistent favorable results in the treatment of actinomycosis and improved the prognosis of the disease during the years following its introduction in 1937. Better results were achieved when these drugs were combined with adjunctive treatment, such as surgical intervention, iodides, or x-rays. Of the sulfonamides, sulfadiazine and sulfisoxasole are the better preparations.[212] Oral doses of 3 to 4 Gm. daily with effective blood levels of 8 to 10 mg. per 100 ml. should be maintained through a period of 4 to 6 months or longer. The usual precautions against possible toxicity must be observed.

Penicillin was introduced in 1945 and has steadily supplanted the sulfonamides. The actinomycete is most sensitive to penicillin in vitro,[213-215] and a host of successful clinical reports testify to the superior efficacy of penicillin. Although in many of these reports penicillin was combined with other therapeutic measures, the antibiotic unquestionably has augmented the rate of recovery, especially in the graver abdominal and thoracic forms. There are still treatment failures, however, in spite of the demonstrated sensitivity of the organism to the antibiotic. The very nature of the disease process with its excessive formation of granulomatous and fibrous tissues impedes the effective concentration of the drug in the diseased area. This necessitates the administration of large doses for prolonged periods and the aid afforded by adjunctive therapy, such as surgical measures and iodides. Another suggested reason for these treatment failures is the penicillin resistances of certain bacteria like *Actinobacillus actinomycetem-comitans* comprising part of the symbiotic flora responsible for the concomitant infection in actinomycosis.

The recommended average dose of peni-

cillin is 3 to 6 million units daily for at least 3 months, extending even to 12 to 18 months, depending upon the response of the patient.

The need for another effective drug that can be used in place of penicillin has been felt for several reasons: (1) the cases that appear to be resistant to penicillin treatment, (2) the increasing incidence of sensitization to penicillin, and (3) the objections to repeated parenteral injections over long periods of time. The newer broad spectrum antibiotics and other chemotherapeutic drugs have been investigated and successfully used. They include streptomycin, tetracycline, oxytetracycline, chlortetracycline, erythromycin, chloramphenicol, isoniazid, and stilbamidine. It is still quite early to confirm the exact value of these drugs. Erythromycin, at least in vitro, is the most effective. Their main disadvantages, especially those of the broad-spectrum antibiotics, are their toxicity and their high cost as compared with penicillin.

Surgical measures are generally admitted to be of considerable value, if not curative as in localized lesions. The procedures range from simple aspiration of fluid to radical complete excision of diseased organs, as pneumonectomy, nephrectomy, or resection of intestines. Better results are obtained when these are associated with drug therapy.

Only a small number of reported cases of actinomycosis were managed with a single mode of therapy. Most were given multiple treatments with preference for penicillin, combined with sulfonamides or any one of the other antibiotics and chemotherapeutic drugs, iodides, x-rays, and surgical procedures. Because of strain variation[207,214,216] the desirability of sensitivity tests performed on the actinomycetes and the symbiotic bacteria isolated from the actinomycotic lesions to determine the proper drugs to be administered has been stressed.

Nocardiosis

Nocardiosis is closely akin to actinomycosis clinically and mycologically, but with enough differences to separate it from the latter condition as a distinct entity. The causative organisms belong to the Nocardia genus which is widely distributed in nature and frequently isolated from soil. There are at least five species known to be pathogenic to man,[217,218] including *Nocardia asteroides, Norcardia brasiliensis, Norcardia madurae,* and *Nocardia pelletieri.* By far the most common is *Nocardia asteroides.* These organisms are gram positive, variably acid fast branching filaments, readily fragmenting into bacillary and coccoid forms. They grow aerobically.

The disease is world-wide in distribution with no racial preference. The source of infection is exogenous. The most frequent portal of entry is the respiratory tract and, less commonly, the injured skin and the gastrointestinal tract. Thus, many of the cases (about 75 per cent) reveal pulmonary involvement. The pulmonary form presents the clinical picture of acute or chronic pneumonitis, with a propensity for hematogenous dissemination to other organs, particularly the central nervous system. Once the central nervous system becomes involved, which occurs in about 30 per cent of the cases, the pulmonary complaints are overshadowed by signs and symptoms simulating brain tumor, brain abscess, or meningitis, and the prognosis becomes graver. The disease may be acute and fulminating, causing death in a few days, but generally it tends to be chronic, with remissions and exacerbations lasting for even up to 3 to 4 years. The average duration is 6 months.

A chronic and localized form of nocardiosis involving the skin and subcutaneous tissues constitutes a certain percentage of the incidence of maduromycosis or mycetoma. The granules found in the discharge from the multiple draining sinuses and fistulas are smaller and softer than the "sulfur granules" of actinomycosis. They are colored black, red, orange, or yellow and do not present clublike terminal structures at the periphery.

In many cases the diagnosis of nocardiosis was made only after operation, very late

in the course of the disease, or at the autopsy table. Definite diagnosis can be made only with the laboratory demonstration of the organism.

Treatment. The particular importance of early diagnosis and institution of treatment in nocardiosis has been emphasized, in view of the remarkable clinical efficacy of the sulfonamides, especially sulfadiazine. Most of the unsuccessful clinical trials with sulfonamides were blamed on the failure to establish an etiological diagnosis and start medication before the disease had become disseminated and progressed to an advanced stage. The sensitivity of *Nocardia asteroides* to sulfonamides has been amply confirmed in vitro[219-224] and in vivo[207,261] and by apparent clinical cures achieved. The effective blood serum level of sulfadiazine is 10 to 20 mg. per 100 ml. with daily oral doses of 6 to 10 Gm. maintained over prolonged periods of time from 3 to 6 months or longer.

The sulfonamides have usually been used in conjunction with other measures, such as surgical intervention, x-rays, iodides, thymol, broad-spectrum antibiotics, and even penicillin (which has been shown to be ineffective, in contrast to its success in the management of actinomycosis). In the choice of antibiotics and other chemotherapeutic agents, in vitro sensitivity tests may be misleading, for their results do not seem to correlate with those of clinical experience. A patient with pulmonary nocardiosis described by Rivera[223] failed to respond to tetracycline but improved with chloramphenicol, although in vitro the isolated Nocardia was found to be sensitive to tetracycline and resistant to chloramphenicol. Both Runyon[222] and Halde and Newstrand[216] found streptomycin to be effective in vitro, but inactive in animal experimental infections. Nonetheless, sensitivity tests are still considered useful by the latter authors. In general, the clinical results with antibiotics and other agents like isoniazid, pregnenolone acetate, or stilbamidine without sulfonamides have been variable.

The sulfones have been shown to be active against the Nocardias.[216,225-227] González Ochoa and his group, who have spearheaded these investigations, worked on the premise that Promin, Promizole, and Diasone act in vivo mainly by breaking down into 4,4′-diaminodiphenyl sulfone (DDS). They concentrated on DDS and found it effective, particularly in infections with *Nocardia brasiliensis*. Of 21 patients with 4 year follow-up periods, 15 were cured and 6 showed marked improvement. The treatment was prolonged: at least 2 years of daily oral administration of 200 mg. Premature withdrawal of the drug led to relapses which were much more resistant to subsequent treatment. The drug was also injected locally into the tumor mass or into the fistulas in 2 ml. amounts of 20 per cent solution daily. In spite of the long-term administration, surprisingly few serious toxic reactions appeared, except for secondary anemia which was easily controlled with iron therapy.

Surgical intervention is still definitely indicated in the management of nocardial infections, particularly in the mycetomatous form.[228] Drainage of abscesses and excision of involved tissues are important adjuncts to sulfonamide therapy.

Sporotrichosis

Sporotrichosis is most often a subcutaneous rather than a systemic or deep fungus infection. The disease normally assumes the rather typical clinical appearance of an ulcerating, granulomatous, chancriform lesion, occurring at the site of direct cutaneous inoculation. The development of satellite lymphangitis and nodules occurring along the inflamed lymphatics is also typical of the disease. The marked variations which occasionally occur in the cutaneous and subcutaneous forms of sporotrichosis demand that all clinicians maintain a high index of suspicion for this particular fungus disease. Systemic involvement may develop from *Sporotrichum schenckii* infection from a cutaneous site. Involvement of almost any organ with sporotrichum may

occur. It has been reported in the kidneys, testicles, epididymis, muscles, bones, joints and tendons, lungs, central nervous system, and tracheobronchial lymph nodes.[25]

Sporotrichum schenckii is found with great difficulty on microscopic examination of stained or unstained clinical material. A positive diagnosis of sporotrichosis can be established only by culture of *Sporotrichum schenckii* on suitable media. Fortunately, this organism is not fastidious in its nutritional requirements, and a brownish to blackish, wrinkled, leathery-appearing colony grows out readily on routine Sabouraud's glucose agar at room temperature, while a white, wrinkled, cerebriform, bacteria-like colony develops on the blood agar cultures at 37° C. This latter culture exhibits the budding yeast phase growth of the organism, while the room temperature culture exhibits hyphal growth with clusters of typical small pyriform conidia.

Treatment. The iodides have become well established as the treatment of choice for sporotrichosis of all types.[25] Invariably, a favorable response can be obtained by the oral administration of increasing doses of a saturated solution of potassium iodide. The initial dose should be approximately 5 drops given 3 times a day. This may be increased by one drop per dose, if a rapid increase is desired, or one drop per day otherwise, until the dosage of 40 to 50 drops 3 times a day is attained. This amount should be maintained for at least 2 to 3 weeks beyond the time that complete clinical healing has occurred. The patient should be instructed that clinical improvement is not to be expected until the iodides have been given for 2 to 3 weeks' time. Only rarely is this form of therapy ineffective in producing complete cure of any type of sporotrichosis. It is puzzling that the iodides have such effect on this fungus, for there is no evidence of increased uptake of radioactive iodine at the site of sporotrichotic lesions in the skin. The organism also grows in artificial media containing 10 per cent concentration of potassium iodide.[25] The iodides will not prevent experimental infection of the rat, although they will effect cure of rat sporotrichosis, if continued in adequate dosage.[229]

The aromatic diamidines, and particularly 2-hydroxystilbamidine, deserve consideration in the therapy of systemic sporotrichosis. An excellent in vitro effect against *Sporotrichum schenckii* has been demonstrated,[152,230] and sporotrichosis has successfully been treated with stilbamidine.[231] One case of central nervous system sporotrichosis has been treated with 2-hydroxystilbamidine.[232] This patient was not able to tolerate the aromatic diamidine and died of renal insufficiency coincident with the drug therapy. Necropsy revealed healing lesions of sporotrichosis. We have seen a patient with sporotrichotic involvement of a knee joint who had a relapse following 2-hydroxystilbamidine therapy. This patient was re-treated with amphotericin B in amounts of 50 mg. administered by slow intravenous drip in 500 c.c. of 5 per cent glucose in water at daily intervals. A total dose of 0.5 Gm. was given. Following this therapy, the sporotrichotic arthritis was no longer evident, and cultures of the joint space were negative for *Sporotrichum schenckii.* We have since learned that this patient has suffered a clinical relapse of the joint sporotrichosis. Amphotericin B has a high in vitro inhibiting effect on *Sporotrichum schenckii.*[1,102] It is probable that this antibiotic would be effective in causing prompt cure of the usual type of uncomplicated lymphatic sporotrichosis. It is doubtful that this toxic antibiotic should be given for the routine, benign forms of sporotrichosis.

Candidiasis

Candidiasis is not usually considered one of the deep or systemic mycoses. This disease (which was previously called moniliasis) is caused by members of the genus Candida, usually *Candida albicans.* This yeastlike fungus can be isolated from normal stool, the vagina, the oral mucous membrane, the skin, and even the normal external auditory canal.[233] It is normally the

cause of superficial infection in mucous membranes of the mouth, the vagina, the paronychial skin, as well as the glabrous skin of the warm, intertriginous areas of the body. *Candida albicans* is rarely reported as the cause of truly systemic disease. Its incidence has sharply increased, however, with the increased usage of broad-spectrum antibiotics and the steroid hormones. These two categories of therapeutic agents have increased human susceptibility to systemic infection with *Candida albicans*.[234-237] Since the fungus is often not pathogenic, there is also a problem associated with the clinical interpretation of the laboratory isolation of *Candida albicans* from such clinical sources as sputum, urine, skin and nail scrapings, nasopharyngeal scrapings, and gastric contents. The finding of *Candida albicans* or other species of Candida on blood culture leaves no question as to pathogenicity.[235]

The members of the Candida genus are easily cultured on almost any of the standard bacteriologic agars or media. *Candida albicans* is separated from the other species of Candida by the findings of terminal chlamydospores in the submerged portion of the culture when grown on corn meal agar. The finding of hyphal growth along with the single-budding blastospores in sputum, scrapings of skin or mucous membranes, and urine is quite significant, and probably indicates that this yeastlike fungus has assumed the role of a true pathogen. *Candida albicans* is a pathogenic opportunist and is found as a superimposed infection in individuals with debilitating diseases, pregnancy, uncontrolled diabetes mellitus, the avitaminoses, and, as previously mentioned, those who are receiving long-term steroid or intensive antibiotic therapy. There is no reliable skin test which can be performed to aid in the diagnosis of candidiasis, and even though agglutinins have been demonstrated as part of systemic candidiasis, their presence has not been reliably consistent.

Treatment. Two antifungal antibiotics must be given consideration in the treatment of systemic candidiasis. Nystatin and amphotericin B have both demonstrated excellent in vitro and in vivo effect against *Candida albicans*.[12,35,50,235,237-243] Nystatin, an antibiotic substance obtained from a species of streptomyces, is very effective in curing candidiasis when it is possible to apply the antibiotic directly to the causative fungus. This is quite possible when the patient has infection of the skin or mucocutaneous junction, the mouth or pharynx, the vagina, or the gastrointestinal tract.[237,240,243] Unfortunately, nystatin is very poorly absorbed from the gastrointestinal tract, and it is impossible to obtain adequate blood levels of this drug when given by this route. Nystatin has not proved effective in controlling or curing pulmonary or bronchopulmonary candidiasis or Candida septicemia. There is one report[235] of systemic candidiasis complicating bacterial endocarditis with apparent cure following the use of intramuscular nystatin in the dose of 9.6 million units administered over a 16 day period. Unfortunately, nystatin given intramuscularly causes marked pain, swelling, and abscess formation at the site of injection. This form of the antibiotic is no longer available. However, nystatin is, without question, the drug of choice for candidiasis of any type when it is possible to use the antibiotic in ointment, lotion, mouth wash, wet dressing, suppository, or tablet form.

Amphotericin B administered intravenously must be considered as the probable therapy of choice for pulmonary candidasis and Candida septicemia. Like nystatin, amphotericin B is very poorly absorbed from the gastrointestinal tract when given orally. Also, like nystatin, amphotericin B produces a marked decrease in the number of *Candida albicans* organisms in the gastrointestinal tract, even when it is administered with a broad-spectrum antibiotic such as tetracycline.[12] There is but one report of the use of intravenous amphotericin B in systemic candidiasis. Utz, Treger, McCullough, and Emmons[50] report the use of this antibiotic in 4 cases. Three of the 4 patients had previously undergone major surgical procedures; 2 developed Candida endocar-

ditis following cardiac surgery, and one developed meningeal candidiasis following an operation for the insertion of a Holter valve to relieve a noncommunicating hydrocephalus. The 3 surgical patients died of candidiasis; however, it is doubtful that they received an adequate trial of amphotericin B therapy. In a fourth case reported by the same authors Candida sepsis followed cystoscopy and retrograde pyelogram for a renal calculus. Blood and urine cultures were positive for *Candida albicans.* Complete recovery ensued after the patient was given a total of 110 mg. of amphotericin B over a 4 day period of time. There is good indication that amphotericin B is effective in the most serious forms of candidiasis.

Two other chemotherapeutic agents deserve definite consideration in the treatment of systemic candidiasis. A saturated solution of potassium iodide has been used for many years as an adjunct in the treatment of all types of candidiasis.[25] This should be given orally. The initial dose is 5 drops 3 times a day following meals, with increments of one drop for each dose, until the total dose of 20 drops 3 times a day is reached and maintained. We are dubious of any true benefit to be obtained in systemic candidiasis from potassium iodide therapy. The careful intravenous administration of methylrosaniline chloride (gentian violet) appears still to be worthy of consideration.[244,245] While the topical application of this dye was the chief means of controlling superficial candidiasis prior to the development of nystatin, methylrosaniline chloride is recommended in intravenous dosage of 5 mg. per kilogram of body weight, given every other day for 7 to 10 days. Concentration of the dye should not exceed 0.5 per cent and the solution should be filtered through a Berkefeld or Seitz filter.

A host of other chemotherapeutic agents have been advocated for the treatment of cutaneous and systemic candidiasis. These include such agents as fatty acid, resin complex,[246] the para-aminobenzoic acid esters,[247] vitamin B complex, brilliant cres green, the aromatic diamidines, testosterone, methyltestosterone, testosterone propionate, and even broad-spectrum antibiotics.[248] It should be mentioned, also, that the new antifungal antibiotic, griseofulvin, has shown no in vitro nor in vivo effect against *Candida albicans* or other members of the candida genus. We have had occasion to use griseofulvin in widespread cutaneous candidiasis and have not observed beneficial effect.

Aspergillosis

The aspergilli abound in nature. Palinologic surveys have shown the prevalence of their anemophilic spores in the atmosphere, where they form one of the aerobiologic factors causing inhalant allergy. The fungus is well known as a saprophyte but can be parasitic to plants, birds, and occasionally, mammals. Of the more than 375 species described, only a few have been isolated as pathogens for man: *Aspergillus fumigatus,* by far the most common; *Aspergillus glaucus; Aspergillus flavus; Aspergillus niger;* and *Aspergillus nidulans.*

The fungous growth is mycelial and characterized by a knoblike fruiting body to which numerous conidia are attached. The tissue form is morphologically the same as the saprophytic phase.

For reasons still not known exactly, the fungus occasionally acquires the ability to invade tissues, causing granulomatous lesions in the lungs, skin, external ears, nasal sinuses, nails, central nervous system, eyes, bones, and vagina. The organism may be a primary or secondary invader. The symptomatology varies with the areas involved, such as that of pneumonitis, onychomycosis, otitis externa, or maduromycosis. The most common form is otomycosis, but pulmonary aspergillosis has gained more medical interest. Primary pulmonary aspergillosis is usually regarded as an occupational disease among individuals unduly exposed to the spores. A significant number of cases of pulmonary aspergillosis is superimposed as a secondary infection on other diseases, such as tuberculosis, carcinoma, bronchiectasis, or infarction. The infection is rarely dissem-

inated.[249] The clinical picture is that of tracheobronchitis, bronchopneumonia, tuberculosis, or tumor. The x-ray findings vary considerably and may show a "sunburst effect," evidence of pneumonic or bronchopneumonic consolidation, cavitation, or, rarely, a diagnostic solitary density whose superior border is outlined by a thin, crescent-shaped area of radiotranslucency.

A definitive diagnosis of aspergillosis is difficult to make and cannot be established on clinical grounds alone. It depends on the demonstration of the organism from the clinical material. Even the cultural isolation of the fungus does not answer the question of whether the isolated organism is a pathogenic invader or a contaminant. The diagnostic criteria are repeated isolations of the aspergillus in pure cultures from the lesions and its demonstration histopathologically with the surrounding tissue reaction of the body.

Together with infections due to organisms generally known as saprophytes, like Mucor, Candida, Geotrichum, and Penicillium, aspergillosis has increased in incidence associated with the present-day extensive use of steroids and broad-spectrum antibiotics.[250,251] Sidransky and Friedman[250] made mice highly susceptible to fatal pulmonary aspergillosis by treatment with steroids and antibiotics. The inhaled spores failed to germinate into hyphae in the control mice, but did so readily in the treated animals. The steroids apparently were more responsible.

Treatment. There is no consistently effective medical control. The noted increased incidence of aspergillosis has made the search for the ideal chemotherapeutic agent more imperative. The occasional favorable results obtained with sulfonamides, penicillin, streptomycin, and other antibiotics were attributed mainly to their action on concomitant bacterial infections.

The in vitro sensitivity of *Aspergillus fumigatus* to certain antibiotics and chemotherapeutic agents has occasionally been studied.[252,253] The marked sensitivity of the organism to mercuric iodide red was noted. Penicillin was inactive in concentrations up to 125 μg per milliliter of medium, but inhibited fructification above this concentration. Even at 10 mg. per milliliter of medium, the broad-spectrum antibiotics succeeded only in preventing fructification.

There is a paucity of clinical reports on adequate therapeutic trials in aspergillosis because of the difficulty of establishing a preoperative or antemortem diagnosis. As a result, the data are grossly insufficient and the conclusions as to the value of the drugs tested are at most preliminary.

The aromatic diamidines were tried in a few cases. Riddell[254] stated that, in his experience, inhalation of 2-hydroxystilbamidine solutions was effective in removing *Aspergillus fumigatus* from bronchial secretions. Stevenson[255] reported a case of pulmonary aspergillosis which responded satisfactorily to two courses of 10 intravenous injections of 150 mg. of 2-hydroxystilbamidine given daily. This therapy was followed by a course of oral nystatin. The patient, however, died later with renal failure. A patient with primary pulmonary aspergillosis described by Librach[256] did not respond to treatment with 2-hydroxystilbamidine as well as to iodides, isoniazid, streptomycin, penicillin, and oxytetracycline. Hinson,[252] on finding that *Aspergillus fumigatus* was inhibited in vitro by diamidinodiphenylamine (M & B 938) in a concentration of 1:100,000, tried the drug clinically in a case of pulmonary aspergillosis. The patient showed only slight equivocal improvement and severe toxic hypotensive symptoms with administration of the drug.

The agent being sought for the effective control of aspergillosis most probably will be found among the antifungal antibiotics that are being introduced in increasing numbers. Riddell[254] also mentioned the effective use of nystatin inhalation. Stevenson's[255] patient also received nystatin, 500,000 units given thrice daily for 8 weeks after two courses of 2-hydroxystilbamidine. In the successful treatment of a case of hypopyon keratitis due to *Aspergillus fumigatus,* Mangiaracine[257] believed that ny

statin administered locally and systemically exerted a beneficial effect. Manning and Robertson[258] recently described a case of pyopneumothorax due to a secondary infection with *Aspergillus fumigatus* after a history of treated pulmonary tuberculosis and a bout of secondary pneumococcal infection for which the patient had received a course of tetracycline. Nystatin was administered by intrapleural instillation of 500,000 units daily. The patient received 15.5 million units in 38 days and improved remarkably, with no side effects due to the drug. The organism disappeared from the sputum. A concomitant secondary infection with *Staphylococcus aureus* was controlled with daily intrapleural administration of 0.5 Gm. chloramphenicol.

An antifungal antibiotic that deserves clinical trial in aspergillosis is griseofulvin, which has aroused interest because of its efficacy in the treatment of dermatomycosis by the oral route.[259,260] Its inhibitory effect on filamentous fungi with chitin cell walls which include the aspergilli is most specific.[261] The antibiotic is relatively nontoxic in therapeutic doses. It is not unreasonable to predict that favorable results will be obtained with the use of griseofulvin in aspergillosis, particularly in its otomycotic and onychomycotic forms, in view of its affinity for keratinous tissues.

In the absence of successful medical treatment in aspergillosis, emphasis is laid on the surgical approach. This is mainly excisional in nature, and most reports of apparent cures in aspergillosis deal with the resection of localized lesions, such as aspergillomas.[262-264]

Mucormycosis

Systemic disease produced by members of the order Mucorales is referred to as a mucormycosis, even though members of the genera *Rhizopus, Absidia,* and others, besides the genus Mucor, may cause the disease. The name mucormycosis is quite acceptable when used in this manner. This is fortunately a rare mycotic disease, which usually develops in individuals who have uncontrolled diabetes mellitus. The disease was first reported by Paltauf[265] in 1885. Since that time, there has been a total of 24 cases of mucormycosis reported in the world's literature.[266,267] A complete summary of the reported cases of mucormycosis is available.[266]

The members of the order Mucorales most commonly found to produce mucormycosis are Rhizopus sp. and Mucor sp. Both of these fungi occur as common laboratory contaminants, and are known as "sugar fungi."[268,269] The hyphae of these fungi exhibit few or no septations and possess the ability to break down sugar. It is of interest that they have been found to be most pathogenic in diabetic individuals. They grow at a very rapid rate and, when contamination with Mucor sp. or Rhizopus sp. occurs, it takes but a few days for the aerial growth of the organisms to fill completely all the available space of the culture tube. The tendency toward such rapid growth has been exhibited clinically when these contaminant fungi assume pathogenicity. Extension through the orbit or paranasal sinuses to the cerebral vessels has occurred in the vast majority of cases,[266,269] and in most instances death has occurred within a matter of days from the time symptoms have been reported. The syndrome consisting of diabetes mellitus, ophthalmoplegia, signs of meningoencephalitis, and possibly sinusitis should make one consider the diagnosis of cerebral mucormycosis.[269]

There are no instances of successful attempts at chemotherapy for mucormycosis. A case has recently been reported[266] in which potassium iodide, given during a 48 hour period prior to death, had no beneficial effect. Unfortunately, this disease is usually diagnosed post mortem, and amphotericin B has not been given an adequate trial. This antifungal antibiotic should be given a therapeutic trial in any individual in whom the antemortem diagnosis of mucormycosis has been established. Chick[270] has treated experimentally produced mucormycosis in rabbits with amphotericin B. The drug was found to protect a rabbit

which had been given a standardized lethal dose of Rhizopus spores. Also, the drug apparently inhibited the transformation of spores into hyphae, when injected intravenously. The animals that were sacrificed following such injections were found to be virtually free of evidence of visceral infection. These experiments suggest that amphotericin B may be of great value in the treatment of human mucormycosis.

References

1. Gold, W., Stout, H. A., Pagano, J. F., and Donovick, R.: Amphotericins A and B, Antifungal Antibiotics Produced by a Streptomycete. I. In Vitro Studies, Antibiotics Annual, 1955-1956, New York, 1956, Medical Encyclopedia, Inc., pp. 579-586.
2. Steinberg, B. A., Jambor, W. P., and Suydam, L. O.: Amphotericins A and B: Two New Antifungal Antibiotics Possessing High Activity Against Deep-Seated and Superficial Mycoses, Antibiotics Annual, 1955-1956, New York, 1956, Medical Encyclopedia, Inc., pp. 574-578.
3. Sternberg, T. H., Wright, E. T., and Oura, M.: A New Antifungal Antibiotic, Amphotericin B, Antibiotics Annual, 1955-1956, New York, 1956, Medical Encyclopedia, Inc., pp. 566-573.
4. Vandeputte, J., Wachtel, J. L., and Stiller, E. T.: Amphotericins A and B, Antifungal Antibiotics Produced by a Streptomycete. II. The Isolation and Properties of the Crystalline Amphotericins, Antibiotics Annual, 1955-1956, New York, 1956, Medical Encyclopedia, Inc., pp. 587-591.
5. Dutcher, J. D., Young, M. B., Sherman, J. H., Hibbits, W., and Walters, D. R.: Chemical Studies on Amphotericin B, Antibiotics Annual, 1956-1957, New York, 1957, Medical Encyclopedia, Inc., pp. 866-869.
6. Littman, M. L., Horowitz, P. L., and Swadey, J. G.: Coccidioidomycosis and Its Treatment With Amphotericin B, Am. J. Med. **24**:568-592, 1958.
7. Bartner, E., Zinnes, H., Moe, R. A., and Kulesza, J. S.: Studies on a New Solubilized Preparation of Amphotericin B, Antibiotics Annual, 1957-1958, New York, 1958, Medical Encyclopedia, Inc., pp. 53-58.
8. Louria, D. B.: Some Aspects of the Absorption, Distribution and Excretion of Amphotericin B in Man, Antibiotic Med. **5**:295-301, 1958.
9. McNall, E. G., Halde, C., Newcomer, V. D., and Sternberg, T. H.: A Biological Assay for the Determination of Amphotericin A and B in Biological Fluids, Antibiotics Annual, 1957-1958, New York, 1958, Medical Encyclopedia, Inc., pp. 131-136.
10. Taylor, R. L., Lynch, H. P., Taylor, R. R., and Weiser, O. L.: The Determination of Amphotericin B Serum Levels in Man, Tr. 17th Conference on Chemotherapy of Tuberculosis, 1958, edited by U. S. Veterans Administration, Dept. of Medicine and Surgery, Washington, D. C., pp. 328-332.
11. Vogel, R. A., and Crutcher, J. C.: Studies on the Bioassay and Excretion of Amphotericin in Patients With Systemic Mycoses, Antibiotic Med. **8**:501-506, 1958.
12. Halde, C., Newcomer, V. D., Wright, E. T., and Sternberg, T. H.: An Evaluation of Amphotericin B in Vitro and in Vivo in Mice Against Coccidioides immitis and Candida albicans, and Preliminary Observations Concerning the Administration of Amphotericin B to Man, J. Invest. Dermat. **28**:217-232, 1957.
13. Louria, D. B., Feder, N., and Emmons, C. W.: Amphotericin B in Experimental Histoplasmosis and Cryptococcosis, Antibiotics Annual, 1956-1957, New York, 1957, Medical Encyclopedia, Inc., pp. 870-877.
14. Summary of Information for Clinical Investigators, E. R. Squibb & Sons, Division of Olin Mathieson Chem. Corp., New Brunswick, N. J., Oct. 28, 1955. Quoted by Littman, Horowitz, and Swadey.[6]
15. Littman, M. L.: Preliminary Observations on the Intravenous Use of Amphotericin B, an Antifungal Antibiotic, in the Therapy of Acute and Chronic Coccidioidal Osteomyelitis, Proc. Symposium Coccidioidomycosis, Feb. 11-13, 1957, pp. 86-94.
16. Fungizone for Infusion. Squibb Amphotericin B, E. R. Squibb & Sons, Division of Olin Mathieson Chem. Corp., New Brunswick, N. J., 1957.
17. Dye, W. E.: In Vitro Studies of Combinations of Antifungal and Antituberculosis Drugs, Tr. 16th Conference on Chemotherapy of Tuberculosis, 1957, edited by U. S. Veterans Administration, Dept. of Medicine and Surgery, Washington, D. C., pp. 347-349.
18. Baum, G. L., Rubel, H., and Schwarz, J.: Treatment of Experimental Histoplasmosis, Antibiotics Annual, 1956-1957, New York, 1957, Medical Encyclopedia, Inc., 1957, pp. 878-882.
19. Baum, G. L., Rubel, H., and Schwarz, J.: Treatment of Experimental Histoplasmosis, Antibiotics **7**:477-482, 1957.
20. Baum, G. L., Schwarz, J., and Wang, C. J. K.: Treatment of Experimental Histoplasmosis

With Amphotericin B, A.M.A. Arch Int. Med. **101**:84-86, 1958.

21. Sternberg, T. H., Newcomer, V. D., Wright, E. T., McNall, E. G., Sorensen, L. J., and Reisner, R. M.: The Treatment of Disseminated Coccidioidomycosis With Amphotericin B, Ann. Meet. Am. Acad. Dermat., Chicago, December, 1958.
22. Winn, W. A.: Coccidioidomycosis, J. Chron. Dis. **5**:430-440, 1957.
23. Emmons, C. W.: Isolation of Coccidioides From Soil and Rodents, Pub. Health Rep. **57**:109-111, 1942.
24. Emmons, C. W., and Ashburn, L. L.: Isolation of Haplosporangium parvum and Coccidioides immitis From Wild Rodents: Their Relationship to Coccidioidomycosis, Pub. Health Rep. **57**:1715-1727, 1942.
25. Conant, N. F., Smith, D. T., Baker, R. D., Callaway, J. L., and Martin, D. S.: Manual of Clinical Mycology, Philadelphia, 1954, W. B. Saunders Company.
26. Smith, D. T., and Harrell, E. R.: Fatal Coccidioidomycosis: A Case of Laboratory Infection, Am. Rev. Tuberc. **57**:368-374, 1948.
27. Wilson, J. W., Smith, C. E., and Plunkett, O. A.: Primary Cutaneous Coccidioidomycosis: The Criteria for Diagnosis and a Report of a Case, California Med. **79**:233-239, 1953.
28. Huntington, R. W., Jr.: Morphology and Racial Distribution of Fatal Coccidioidomycosis. Report of a Ten Year Autopsy Series in an Endemic Area, J.A.M.A. **169**:115-118, 1959.
29. Smith, C. E., Saito, M. T., Beard, R. R., Rossenberg, H. G., and Whiting, E. G.: Histoplasmin Sensitivity and Coccidioidal Infection; Occurrence of Cross Reactions, Am. J. Pub. Health **39**:722-736, 1949.
30. Smith, C. E., Saito, M. T., Beard, R. R., Kepp, R. M., Clark, R. W., and Eddie, B. U.: Serological Tests in the Diagnosis and Prognosis of Coccidioidomycosis, Am. J. Hyg. **52**:1-21, 1950.
31. Gephardt, M. C., and Hanlon, T. J.: Treatment of Disseminated Coccidioidomycosis With Stilbamidine (Case History), J. Oklahoma M.A. **47**:55-58, 1954.
32. Snapper, I., Baker, L. A., Edidin, B. D., and Kushner, D. S.: The Results of 2-Hydroxystilbamidine Therapy in Disseminated Coccidioidomycosis, Ann. Int. Med. **43**:271-286, 1955.
33. Gordon, L. E., Smith, C. E., Tompkins, M., and Saito, M. T.: Sensitivity of Coccidioides immitis to 2-Hydroxystilbamidine and the Failure of the Drug in the Treatment of Experimental Coccidioidomycosis, J. Lab. & Clin. Med. **43**:942-945, 1954.
34. Gordon, L. E., and Smith, C. E.: Mycostatin and Aminostilbamidine Treatment of Experimental Coccidioidomycosis. In Sternberg, T. H., and Newcomer, V. D., editors: Therapy of Fungus Diseases, Boston, 1955, Little, Brown & Company, pp. 249-254.
35. Hazen, E. L., and Brown, R.: Fungicidin, an Antibiotic Produced by a Soil Actinomycete, Proc. Soc. Exper. Biol. & Med. **76**:93-97, 1951.
36. Cohen, R., and Webb, P. A.: Nystatin, a Coccidioidocidal Antibiotic, Arch. Pediat. **69**:414-416, 1952.
37. Newcomer, V. D., Wright, E. T., Leeb, A. J., Tarbet, J. E., and Sternberg, T. H.: The Evaluation of Nystatin on the Course of Coccidioidomycosis in Mice, J. Invest. Dermat. **22**:431-440, 1954.
38. Procknow, J. J., and Loosli, C. G.: Treatment of the Deep Mycoses, A.M.A. Arch. Int. Med. **101**:765-802, 1958.
39. Cohen, R.: Diethylstilbestrol, a Coccidioidal Fungicide, Arch. Pediat. **71**:291-292, 1954.
40. Lamb, J. H.: Combined Therapy in Histoplasmosis and Coccidioidomycosis: Methyltestosterone and Meth-dia-sulfonamides, A.M.A. Arch. Dermat. & Syph. **70**:695-712, 1954.
41. Conan, N. J., and Hyman, G. A.: Disseminated Coccidioidomycosis, Treatment With Protoanemonin, Am. J. Med. **9**:408-413, 1950.
42. Wier, R. H., Egeberg, R. O., Lack, A. R., and Leiby, G. M.: A Clinical Trial of Prodigiosin in Disseminated Coccidioidomycosis, Am. J. M. Sc. **224**:70-76, 1952.
43. Fiese, M. J., Radding, J., Cheu, S., and Steinback, O. K.: Disseminated Coccidioidomycosis; Treatment With Ethyl Vanillate; a Preliminary Report, California Med. **80**:349-356, 1954.
44. Cohen, R.: Four New Fungicides for Coccidioides immitis; Sodium Caprylate, Ethyl Vanillate, Fradicin, Thiolutin, Arch. Pediat. **68**:259-264, 1951.
45. Fiese, M. J.: Treatment of Disseminated Coccidioidomycosis With Amphotericin B, California Med. **86**:119-120, 1957.
46. Klapper, M. S., Smith, D. T., and Conant, N. F.: Disseminated Coccidioidomycosis Apparently Cured With Amphotericin B, J.A.M.A. **167**:463-466, 1958.
47. Williams, R. M., and Skipworth, G. B.: Treatment of Disseminated Coccidioidomycosis With Amphotericin B, A.M.A. Arch. Dermat. **78**:97-100, 1958.
48. Hunter, R. C., and Mongan, E. S.: Disseminated Coccidioidomycosis Treated With Amphotericin B., U.S. Armed Forces M.J. **9**:1474-1486, 1958.
49. Horowitz, I., and Berglund, E. M.: Amphotericin B: A New Antifungal Antibiotic in the Treatment of Coccidioidomycosis, Tr. 16th Conference on Chemotherapy in Tuberculosis, 1957, edited by U.S. Veterans Administration,

Dept. of Medicine and Surgery, Washington, D. C., pp. 345-347.

50. Utz, J. P., Treger, A., McCullough, N. B., and Emmons, C. W.: Amphotericin B: Intravenous Use in 21 Patients With Systemic Fungal Diseases, Antibiotics Annual, 1958-1959, New York, 1959, Medical Encyclopedia, Inc., pp. 628-634.
51. Sorensen, L. J., McNall, E. G., and Sternberg, T. H.: The Development of Strains of Candida albicans and Coccidioides immitis Which Are Resistant to Amphotericin B, Antibiotics Annual, 1958-1959, New York, 1959, Medical Encyclopedia, Inc., pp. 920-923.
52. Edwards, P. Q., and Klaer, J. H.: World-wide Geographic Distribution of Histoplasmosis and Histoplasmin Sensitivity, Am. J. Trop. Med. & Hyg. **5**:235-257, 1956.
53. Manos, N. E., Ferebee, S. H., and Kerschbaum, W. F.: Geographic Variation in the Prevalence of Histoplasmin Sensitivity, Dis. Chest **29**:649-668, 1956.
54. Larsh, H. W., Hinton, A., and Cozad, G. C.: Natural Reservoir of Histoplasma capsulatum, Am. J. Hyg. **63**:18-27, 1956.
55. Brasher, C. A., and Furcolow, M. L.: Problems in Treatment of Chronic Histoplasmosis as Experienced in Over Twenty Cases. In Sternberg, T. H., and Newcomer, V. D., editors: Therapy of Fungus Diseases, Boston, 1955, Little, Brown & Company, pp. 287-288.
56. Furcolow, M. L.: The Clinical Diagnosis of Histoplasmosis, Postgrad. Med. **20**:349-364, 1956.
57. Wilson, J. W.: Clinical and Immunological Aspects of Fungous Diseases, Springfield, Ill., 1957, Charles C Thomas, Publisher.
58. Lehan, P. H., and Furcolow, M. L.: Epidemic Histoplasmosis, J. Chron. Dis. **5**:489-503, 1957.
59. Baum, G. L., Adriano, S. M., and Schwarz, J.: Effect of Cortisone on Experimental Histoplasmosis in Mice, Am. J. Clin. Path. **24**:903-909, 1954.
60. Mankowski, Z. T., and Littleton, B. J.: Action of Cortisone and ACTH on Experimental Fungus Infections, Antibiotics **4**:253-258, 1954.
61. Koenigsbauer, H.: Ueber die Wirkung von ACTH und Corton auf die experimentelle Histoplasmose der Ratte, Zentralbl. Bakt. **159**: 473-476, 1953.
62. Vogel, R. A., Michael, M., and Timpe, A.: Cortisone in Experimental Histoplasmosis, Am. J. Path. **31**:535-543, 1955.
63. Silverman, F. N., Schwarz, J., Lahey, M. E., and Carson, R. P.: Histoplasmosis, Am. J. Med. **19**:410-459, 1955.
64. Fox, H.: Exfoliative Dermatitis Complicated by Fatal Acute Disseminated Histoplasmosis, A.M.A. Arch. Dermat. & Syph. **68**:734-737, 1953.
65. Young, R. V., Cleve, E. A., and Vicente-Mastellari, A.: Acute Pulmonary Histoplasmosis on the Isthmus of Panama, A.M.A. Arch. Int. Med. **100**:430-435, 1957.
66. Michael, M., and Vogel, R. A.: Histoplasmosis. Report of a Case, With Observations on Management, New England J. Med. **251**:884-887, 1954.
67. Fissel, G. E.: Acute Fulminating Histoplasmosis, Am. J. Roentgenol. **76**:60-63, 1956.
68. Tegeris, A. S., and Smith, D. T.: Acute Disseminated Pulmonary Histoplasmosis Treated With Cortisone and MRD-112, Ann. Int. Med. **48**:1414-1420, 1958.
69. Schwartz, B.: Histoplasmosis of the Lungs, A.M.A. Arch. Int. Med. **94**:970-994, 1954.
70. Packard, J. S., Finkelstein, H., and Turner, W. E.: Acute Pulmonary Histoplasmosis. Treatment With Cortisone, A.M.A. Arch. Int. Med. **99**:370-375, 1957.
71. Matthiesen, D. E.: The Surgical Significance of Solitary Circumscribed Lung Nodules in Histoplasmosis. Report of Three Cases, Am. Rev. Tuberc. **69**:829-836, 1954.
72. Polk, J. W., Cubiles, J. A., and Buckingham, W. W.: The Surgical Treatment of Chronic Progressive Histoplasmosis, J. Thoracic Surg. **34**:341-343, 1957.
73. Puckett, T. F.: Pulmonary Histoplasmosis, Am. J. Surg. **90**:92-100, 1955.
74. Vivian, D. N., Weed, L. A., McDonald, J. R., Clagett, O. T., and Hodgson, C. H.: Histoplasmosis: Clinical and Pathologic Study of 20 Cases, Surg. Gynec. & Obst. **99**:53-62, 1954.
75. Christie, A., Middleton, J. G., Peterson, J. C., and McVickar, D. L.: Treatment of Disseminated Histoplasmosis With Ethyl Vanillate, Pediatrics **7**:7-18, 1951.
76. Burton, C. T., and Wallenborn, P. A.: Histoplasmosis of the Larynx, Virginia M. Month. **80**:665-668, 1953.
77. Ellis, F. F., Scott, R. J., and Miller, J. M.: Treatment of Progressive Histoplasmosis With Ethyl Vanillate and Propamidine, Antibiotics **2**:347-350, 1952.
78. Locket, S., Atkinson, E. A., and Grieve, W. S. M.: Histoplasmosis in Great Britain. Description of a Second Case of Disseminated Histoplasmosis; Treatment by Ethyl Vanillate, Brit. M. J. **2**:857-860, 1953.
79. Middleton, J. W.: Pulmonary Histoplasmosis, J. Louisiana M. Soc. **107**:1-6, 1955.
80. Shull, H. J.: Human Histoplasmosis: A Disease With Protean Manifestations Often With Digestive System Involvement, Gastroenterology **25**:582-595, 1953.
81. Sones, C. A., Rotkow, M. J., and Dunn, R. C.: Acute Disseminated Histoplasmosis; Report of

a Case in ... dult, J. Iowa M. Soc. **45**:463-468, 1...

82. Zinn... H., and Hall, W. H.: Chronic ... d Laryngeal Histoplasmosis Successfu... ...ted With Ethyl Vanillate, Minnesota Med. **36**:249-252, 1953.
83. Egbert, E., Johnson, J. E., Tsai, S. Y., and Levin, W. C.: Recent Experiences With Histoplasmosis, Texas J. Med. **51**:792-798, 1955.
84. Sutliff, W. D.: Experience With the Course and Chemotherapy of Chronic Pulmonary Histoplasmosis, Am. Rev. Tuberc. **75**:912-920, 1957.
85. Ludwig, K. A., Murray, F. J., Smith, J. K., Thompson, C. R., and Werner, H. W.: Laboratory Studies on β-diethylaminoethyl fencholate, Antibiotics **4**:56-61, 1954.
86. Ellis, M., and Callander, R. J.: Treatment of Histoplasmosis With MRD-112: Report of a Case, J. Tennessee M. A. **48**:411-415, 1955.
87. Furcolow, M. L., and Brasher, C. A.: Trials With 2-hydroxystilbamidine, Aminostilbamidine, MRD-112 and Other Agents in Pulmonary Histoplasmosis; Experiments With More Than 20 Cases, In Sternberg, T. H., and Newcomer, V. D., editors: Therapy of Fungus Diseases, Boston, 1955, Little, Brown & Company, pp. 289-291.
88. Fitzpatrick, M. J., and Reuber, M. D.: Addison's Disease Associated With Disseminated Histoplasmosis and Pulmonary Tuberculosis, Am. Rev. Tuberc. **72**:675-684, 1955.
89. Terry, R. T., and Matthews, J. H.: Histoplasmosis: Treatment of Two Cases with β-diethylaminoethyl fencholate, Dis. Chest **30**:570-575, 1956.
90. Polk, J. W., Brasher, C. A., de Castro, J., and Buckingham, W. W.: The Surgical Treatment of Pulmonary Histoplasmosis With an Evaluation of MRD-112 as a Possible Adjunct, J. Thoracic Surg. **31**:148-164, 1956.
91. Lehan, P. H., Yates, J. L., Brasher, C. A., Larsh, H. W., and Furcolow, M. L.: Experience With the Therapy of Sixty Cases of Deep Mycotic Infections, Dis. Chest **32**:597-614, 1957.
92. Mayer, R. L., Eisman, P. C., Geftic, S., Konopka, E., and Tanzola, J.: Sulfonamides and Experimental Histoplasmosis, Antibiotics **6**:215-225, 1956.
93. Louria, D. B., and Feder, N.: Sulfonamides in Experimental Histoplasmosis, Antibiotics **7**:471-476, 1957.
94. Christie, A.: The Disease Spectrum of Human Histoplasmosis, Ann. Int. Med. **49**:544-555, 1958.
95. Niño, F. L.: Nueva observacion de histoplasmosis en la Republica Argentina, Mycopath. et mycol. appl. **5**:128-132, 1951.
96. Halperin, A., Pierini, D. O., Mortola, G., and Acrich, M.: Pleuresia serofibrinosa. Iniciación aparente de una histoplasmosis generalizada, Prensa méd. argent. **41**:575-582, 1954.
97. Esandi, F., and Chicote, M. N.: Histoplasmosis localizada, Prensa méd. argent. **42**:1610-1612, 1955.
98. Cordero, A. A.: Histoplasmosis, Prensa méd. argent. **43**:1074-1079, 1956.
99. Negroni, P.: Nuevos agentes en el tratamiento de las micosis, Rev. Asoc. méd. argent. **71**: 298-304, 1957.
100. Wail, M.: Histoplasmosis, Tórax **6**:398-401, 1957.
101. MacKinnon, J. E.: Revision critica de la investigación y de la literatura micologica en el Uruguay en el periodo 1946-1956, Mycopath. et mycol. appl. **9**:224-240, 1958.
102. Larsh, H. W., Silberg, S. L., and Hinton, A.: Use of the Tissue Culture Method in Evaluating Antifungal Agents, Antibiotics Annual, 1956-1957, New York, 1957, Medical Encyclopedia, Inc., pp. 918-922.
103. Drouhet, E.: Action de l'amphotéricin B dans l'histoplasmose africaine à grandes formes, Bull. Soc. path. exot. **51**:76-82, 1958.
104. Greendyke, R. M., and Kaltreider, N. L.: Chronic Histoplasmosis; Report of a Patient Successfully Treated With Amphotericin B, Am. J. Med. **26**:135-138, 1959.
105. Heyn, R. M., and Giammona, S. T.: Disseminated Histoplasmosis Treated With Amphotericin B, A.M.A. J. Dis. Child. **98**:253-256, 1959.
106. Jarniou, A. P., Kerbrat, G., Moreau, A., Duval, and Drouhet, E.: Histoplasmose pulmonaire africaine avec suppuration diffuse apparue après une année d'évolution, Bull. Soc. méd. hôp. Paris **74**:918-927, 1958.
107. Little, J., Bruce, J., Andrews, H., Crawford, K., and McKinley, G.: Treatment of Disseminated Infantile Histoplasmosis With Amphotericin B, Pediatrics **24**:1-6, 1959.
108. Miller, J. M., Ginsberg, M., Johnson, H. R., and Bogosian, A.: The Treatment of Histoplasmosis With Amphotericin B (Fungizone), Antibiotic Med. **5**:593-597, 1958.
109. Rubin, H., Lehan, P. H., and Furcolow, M. L.: Severe Non-fatal Histoplasmosis, New England J. Med. **257**:599-602, 1957.
110. Utz, J. P., Louria, D. B., Feder, N., Emmons, C. W., and McCullough, N. B.: A Report of Clinical Studies on the Use of Amphotericin in Patients With Systemic Fungal Diseases, Antibiotics Annual, 1957-1958, New York, 1958, Medical Encyclopedia, Inc., pp. 65-70.
111. Schwarz, J., and Goldman, L.: Epidemiological Study of North American Blastomycosis, A.M.A. Arch. Dermat. & Syph. **71**:84-88, 1955.

112. Smith, J. G., Jr., Harris, J. S., Conant, N. F., and Smith, D. T.: An Epidemic of North American Blastomycosis, J.A.M.A. **155**:641-646, 1955.

113. Martin, D. S., and Smith, D. T.: Blastomycosis (American Blastomycosis, Gilchrist's Disease). I. Review of Literature. II. A Report of Thirteen New Cases, Am. Rev. Tuberc. **39**: 275-304, 488-515, 1939.

114. Harris, J. S., Smith, J. G., Humbert, W. C., Conant, N. F., and Smith, D. T.: North American Blastomycosis in an Epidemic Area, Pub. Health Rep. **72**:95-100, 1957.

115. Kunkel, W. M., Jr., Weed, L. A., McDonald, J. R., and Clagett, O. T.: North American Blastomycosis—Gilchrist's Disease: a Clinicopathologic Study of Ninety Cases, Surg. Gynec. & Obst. **99**:1-26, 1954.

116. Schwarz, J., and Baum, G. L.: Blastomycosis, Am. J. Clin. Path. **21**:999-1029, 1951.

117. Wilson, J. W., Cawley, E. P., Weidman, F. D., and Gilmer, W. S.: Primary Cutaneous North American Blastomycosis, A.M.A. Arch. Dermat. & Syph. **71**:39-45, 1955.

118. Harrell, E. R., and Curtis, A. C.: North American Blastomycosis, Am. J. Med. (In press.)

119. Smith, D. T.: Immunologic Types of Blastomycosis: A Report of 40 Cases, Ann. Int. Med. **31**:463-469, 1949.

120. Snapper, I., Schneid, B., McVay, L., and Lieben, F.: Pharmacology and Therapeutic Value of Diamidine Derivatives—Particularly of 2-Hydroxystilbamidine, Tr. New York Acad. Sc. **14**:269-275, 1952.

121. Sutliff, W. D., Kyle, J. W., and Hobson, J. L.: North American Blastomycosis: Clinical Forms of the Disease and Treatment With Stilbamidine and 2-Hydroxystilbamidine, Ann. Int. Med. **41**:89-107, 1954.

122. Curtis, A. C., and Harrell, E. R.: Use of Two Stilbene Derivatives (Diethylstilbestrol and Stilbamidine) in Treatment of Blastomycosis, A.M.A. Arch. Dermat. & Syph. **66**:676-690, 1952.

123. Fink, J. C., Vanderploeg, D. E., and Moursund, M. P.: Stilbamidine in the Treatment of Cutaneous Blastomycosis, J.A.M.A. **151**:1395-1397, 1953.

124. Pariser, H., Levy, E. D., and Rawson, A. J.: Treatment of Blastomycosis With Stilbamidine, J.A.M.A. **152**:129-130, 1953.

125. Smith, D. T.: Stilbamidine Therapy in Blastomycosis, GP **8**:69-76, 1953.

126. Snapper, I., and McVay, L. V.: Treatment of North American Blastomycosis With 2-Hydroxystilbamidine, Am. J. Med. **15**:603-623, 1953.

127. Schoenbach, E. R., and Greenspan, E. M.: The Pharmacology, Mode of Action and Therapeutic Potentialities of Stilbamidine, Pentamidine, Propamidine and Other Aromatic Diamidines—a Review, Medicine **27**:327-377, 1948.

128. Cherniss, E. I., and Waisbren, B. A.: North American Blastomycosis: A Clinical Study of 40 Cases, Ann. Int. Med. **44**:105-123, 1956.

129. Curtis, A. C., and Bocobo, F. C.: North American Blastomycosis, J. Chron. Dis. **5**:404-429, 1957.

130. Harrell, E. R., Bocobo, F. C., and Curtis, A. C.: A Study of North American Blastomycosis and Its Treatment With Stilbamidine and 2-Hydroxystilbamidine, Ann. Int. Med. **43**:1076-1091, 1955.

131. Kuhn, B. H.: Stilbamidine-Resistant North American Blastomycosis, A.M.A. Arch Dermat. **73**:556-559, 1956.

132. Harrell, E. R., and Curtis, A. C.: The Treatment of North American Blastomycosis With Amphotericin B, A.M.A. Arch. Dermat. **76**: 561-569, 1957.

133. Derbes, V. J., and Krafchuck, J. D.: Response of North American Blastomycosis to Amphotericin B, Bull. Tulane M. Fac. **17**:157-163, 1958.

134. Cornbleet, T.: Thyroid-Iodide Therapy of Blastomycosis, A.M.A. Arch. Dermat. **76**:545-548, 1957.

135. Goetz, H.: Klinische und experimentelle Studien ueber das Granuloma para-coccidioides (Morbus Lutz-Splendore-de Almeida), Arch. Dermat. u. Syph. **198**:507-528, 1954.

136. Molese, A., Pane, A., Vingiani, A., and Pagano, A.: Su un caso di granulomatosi paracoccidioidea; malattia di Lutz-Splendore-Almeida, Riforma Med. **70**:1009-1014, 1956.

137. Paltauf, R.: Lung: Blastomycosis (Brazilian), Brooklyn Hosp. J. **11**:157-159, 1953.

138. Perry, H. O., Weed, L. A., and Kierland, R. R.: South American Blastomycosis, A.M.A. Arch. Dermat. & Syph. **70**:477-482, 1954.

139. Lacaz, C. da S.: South American Blastomycosis, An. Fac. med. Univ. São Paulo **29**:1-20, 1955-1956.

140. Sampaio, S. A. P., and de Almeida, F.: O vanilato de etila no tratamento de algumas micoses, Arq. hyg. e saúde púb. **18**:295-296, 1953.

141. Lacaz, C. da S., del Negro, G., and Bolognani, H., Jr.: Ação da Fungicidina sôbre o Paracoccidioides brasiliensis. Resultados preliminares em 3 doentes de blastomicose brasiliensis, Rev. Hosp. clín. São Paulo **10**:436-439, 1955.

142. Floch, H., and Saccharin, H.: Sur un cas de maladie de Lutz, Bull. Soc. path. exot. **48**:686-691, 1955.

143. Lacaz, C. da S., Sampaio, S.A.P., and del Negro, G.: Atividade de duas diamidinas aromáticas na blastomicose sul-americana, Hospital, Rio de Janeiro **48**:163-169, 1955.

144. MacKinnon, J. E., Sanjines, A., and Artagaveytia-Allende, R. C.: Quimioterapia de la blastomicose sudamericana; ensayo in vitro de cuatro diamidinas aromáticas, dos sulfamidas, una sulfona, y un antihistamínicio; ensayo clínico de la diamidinodifenilamina, An. Fac. med. Montevideo **42**:131-142, 1957.
145. Borelli, D., and Rodriguez, G.: Blastomycosis Treated With Diamidinodiphenylamine, Tr. Roy. Soc. Trop. Med. & Hyg. **52**:289, 1958.
146. Lacaz, C. da S., and Sampaio, S.A.P.: Tratamento da blastomicose sul-americana com Anfotericina B. Resultados preliminares, Rev. paulista med. **52**:443-450, 1958.
147. Ajello, L.: Occurrence of Cryptococcus neoformans in Soils, Am. J. Hyg. **67**:7[illegible] 1958.
148. Emmons, C. W.: Saprophytic Soi[illegible]ryptococcus neoformans Associate[illegible]he Pigeon (Columba livia), Am. J. Hyg. **6**[illegible]27-232, 1955.
149. Jacobs, L. G.: Pulmonary Torulosis, Radiology **71**:398-403, 1958.
150. Beeson, P. B.: Cryptococcic Meningitis of Nearly Sixteen Years' Duration, A.M.A. Arch. Int. Med. **89**:797-801, 1952.
151. Evans, E. E., and Harrell, E. R.: Cryptococcosis (Torulosis): A Review of Recent Cases, Univ. Michigan M. Bull. **18**:43-63, 1952.
152. Bocobo, F. C. Curtis, A. C., and Harrell, E. R.: In Vitro Fungistatic Activity of Stilbamidine, Propamidine, Pentamidine and Diethylstilbestrol, J. Invest. Dermat. **21**:149-156, 1953.
153. Taschdjian, C. L.: In Vitro Effects of Stilbamidine and 2-Hydroxystilbamidine on Some Pathogenic Fungi, J. Invest. Dermat. **22**:521-526, 1954.
154. Fisher, A. M.: The Clinical Picture Associated With Infections Due to Cryptococcus neoformans (Torula histolytica). Report of Three Cases With Some Experimental Studies, Bull. Johns Hopkins Hosp. **86**:383-414, 1950.
155. Miller, J. M., Smith, G. W., and Headley, W. H.: Treatment of Cryptococcus neoformans Infection in Mice With Stilbamidine, Science **118**:31-33, 1953.
156. Solotorovsky, M., Quabeck, G., and Winsten, S.: Antifungal Activity of Candidin, Nystatin, Eulicin, and Stilbamidine Against Experimental Infections in the Mouse, Antibiotics **8**:364-371, 1958.
157. Leithold, S. L., Reeder, P. S., and Baker, L. A.: Cryptoccocal Infection Treated With 2-Hydroxystilbamidine in a Patient With Boeck's Sarcoid, A.M.A. Arch. Int. Med. **99**:736-743, 1957.
158. Whitehill, M. R., and Rawson, A. J.: Treatment of Generalized Cryptococcosis With 2-Hydroxystilbamidine. Report of a Case With Apparent Cure, Virginia M. Month. **81**:591-594, 1954.
159. Ferguson, G. B.: Mycotic Diseases of the Lower Respiratory Tract, Ann. Otol. Rhin. & Laryng. **64**:1245-1260, 1955.
160. Warren, E. A., Stephens, G. G., and Crow, E. W.: Cryptococcosis; Treatment of Two Cases With Stilbamidine, J. Kansas M. Soc. **58**:301-303, 1957.
161. Miller, J. M., Schoenbach, E. B., Long, P. H., Shuttleworth, J. S., and Snider, G. E.: Treatment of Infections Due to Cryptococcus neoformans With Stilbamidine, Antibiotics **2**:444-446, 1952.
162. Bacon, A. E., Scott, E. G., and Huntington, P. W.: Meningoencephalitis Due to Cryptococcus neoformans, Delaware M. J. **26**:3-8, 1954.
163. Willis, J. D., Marples, M. J., DiMenna, M. E., Rodda, R., and Pullar, T. H.: Cryptococcal (Torula) Meningitis in New Zealand; a Report of Four Cases, New Zealand M. J. **56**:99-109, 1957.
164. Brown, R., and Hazen, E. L.: Nystatin and Actidione: Two Antifungal Agents Produced by Streptomyces noursei. In Sternberg, T. H., and Newcomer, V. D., editors: Therapy of Fungus Diseases, Boston, 1955, Little, Brown & Company, pp. 164-167.
165. Bonfiglioli, H., and Longobardi, A. L.: Sensibilidad in vitro de Cryptococcus neoformans a diversos antibioticos y quimioterápicos, Prensa méd. argent. **44**:2657-2659, 1957.
166. Brown, R., and Hazen, E. L.: Present Knowledge of Nystatin, an Antifungal Antibiotic, Tr. New York Acad. Sc. **19**:447-456, 1957.
167. Littman, M. L., and Zimmerman, L. E.: Cryptococcosis, Torulosis or European Blastomycosis, New York, 1956, Grune & Stratton, Inc.
168. Mulsow, F. W., and Lindley, E. L.: Cryptococcus neoformans Meningoencephalitis: A Case Report, J. Iowa M. Soc. **47**:579-580, 1957.
169. Walz, D. V., Hasenclever, H. F., and McKee, A. P.: A Dual Human Infection With Candida albicans and Cryptococcus neoformans, Am. J. Clin. Path. **26**:794-798, 1956.
170. Hoigne, R., Beer, K., and Cottier, H.: Ueber Torulose, Schweiz. med. Wchnschr. **87**:97-101, 1957.
171. Appelbaum, E., and Shtokalko, S.: Cryptococcus Meningitis Arrested With Amphotericin B, Ann. Int. Med. **47**:346-351, 1957.
172. Perruchio, P., Bruel, R., Lagarde, C., and Delpy, J.: Le torulome bronchiectasiant; une nouvelle forme clinique de la torulose respiratoire, Presse méd. **67**:387-390, 1959.
173. Carton, C. A., and Liebig, C. S.: Treatment of Central Nervous System Cryptococcosis. Laboratory Studies, A.M.A. Arch. Int. Med. **91**:773-783, 1953.
174. Kligman, A. M., and Weidman, F. D.: Experimental Studies on Treatment of Human

Torulosis, A.M.A. Arch. Dermat. & Syph. **60**:726-741, 1949.

175. Wilson, H. M., and Duryea, A. W.: Cryptococcus Meningitis (Torulosis) Treated With a New Antibiotic, Actidione, A.M.A. Arch. Neurol. & Psychiat. **66**:470-480, 1951.
176. Alajouanine, T., Houdart, R., and Drouhet, E.: Les formes chirurgicales spinales de la torulose; torulome de la queue de cheval, Rev. neurol. **88**:153-163, 1953.
177. Farrell, A., and Helfer, L. M.: Cryptococcus neoformans Meningoencephalitis. Report of Case Treated With Actidione and Forced Drainage, Texas J. Med. **51**:516-518, 1955.
178. Carton, C. A.: Treatment of Central Nervous System Cryptococcosis: A Review and Report of Four Cases Treated With Actidione, Ann. Int. Med. **37**:123-154, 1952.
179. Boshes, L. D., Sherman, I. C., Hesser, C. J., Milzer, A., and MacLean, H.: Fungus Infections of the Central Nervous System; Experience in Treatment of Cryptococcosis With Cycloheximide (Actidione), A.M.A. Arch. Neurol. & Psychiat. **75**:175-197, 1956.
180. Buckle, G., Curtis, D. R., and McKeon, J. M.: Therapy of Human Torulosis With Actidione and "Contramine": A Report on Two Cases, M. J. Australia **2**:854-859, 1955.
181. Caldwell, D. C., and Raphael, S. S.: A Case of Cryptococcal Meningitis, J. Clin. Path. **8**:32-37, 1955.
182. Geaney, B., Horsfall, W. R., and Neilson, G.: Torulosis in Queensland: Report of Fourteen Cases, M. J. Australia **2**:378, 1956.
183. Markham, J. W., Alcott, D. L., and Manson, R. M.: Cerebral Granuloma Caused by Cryptococcus neoformans; Report of a Case, J. Neurosurg. **15**:562-568, 1958.
184. Moore, M.: Cryptococcosis With Cutaneous Manifestations. Four Cases With a Review of Published Reports, J. Invest. Dermat. **28**:159-182, 1957.
185. Sherman, I. C., and Boshes, L. D.: Some Fungous Infections of the Central Nervous System: Experience With Recent Treatments, Bol. Asoc. méd. Puerto Rico **48**:225-238, 1956.
186. Bakerspigel, A., Campsall, E. W. R., and Hession, B. L.: A Case of Cryptococcal Meningitis in Southwestern Ontario, Canad. M.A.J. **79**:998-1002, 1958.
187. Biddle, A., and Koenig, H.: An Agent Effective Against Cryptococcosis of the Central Nervous System, A.M.A. Arch. Int. Med. **102**:801-805, 1958.
188. Burrows, B., and Barclay, W. R.: Combined Cryptococcal and Tuberculous Meningitis Complicating Recticulum Cell Sarcoma, Am. Rev. Tuberc. **78**:760-768, 1958.
189. Fitzpatrick, M. J., Rubin, H., and Poser, C. M.: The Treatment of Cryptococcal Meningitis With Amphotericin B, a New Antifungal Agent, Ann. Int. Med. **49**:249-259, 1958.
190. Furcolow, M. L., and Rubin, H.: Promising Results in Cryptococcal Meningitis, Tr. 17th Conference on Chemotherapy of Tuberculosis 1958, edited by U.S. Veterans Administration, Dept. of Medicine and Surgery, Washington, D. C., pp. 309-310.
191. Gantz, J. A., Metzel, J. A., and Keller, L. B.: Cryptococcal Meningitis Treated With Amphotericin B, A.M.A. Arch. Int. Med. **102**:795-800, 1958.
192. Gettelfinger, W. C.: Cryptococcus Meningitis, J. Kentucky M. A. **56**:1112-1113, 1958.
193. Lerner, A. B., and Crounse, R. G.: Cutaneous Cryptococcosis Without Apparent Systemic Involvement, A.M.A. Arch. Dermat. **77**:457, 1958.
194. Rubin, H., Lehan, P. H., Fitzpatrick, M. J., and Furcolow, M. L.: Amphotericin B in the Treatment of Cryptococcal Meningitis, Antibiotics Annual, 1957-1958, New York, 1958, Medical Encyclopedia, Inc., pp. 71-73.
195. Smith, G. W.: The Treatment of Torula Meningoencephalitis With Amphotericin B, J. Neurosurg. **15**:572-575, 1958.
196. Rubin, H., and Furcolow, M. L.: Promising Results in Cryptococcal Meningitis, Neurology **8**:590-595, 1958.
197. Martin, W. J., Nichols, D. R., Svien, H. J., and Ulrich, J. A.: Cryptococcosis. Further Observations and Experiences With Amphotericin B, A.M.A. Arch. Int. Med. **104**:4-14, 1959.
198. Kuykendall, S. J., Ellis, F. H., Jr., Weed, L. A., and Donoghue, F. E.: Pulmonary Cryptococcosis, New England J. Med. **257**:1009-1016, 1957.
199. Bonmati, J., Rogers, J. V., Jr., and Hopkins, W. A.: Pulmonary Cryptococcosis, Radiology **66**:188-194, 1956.
200. Cornish, A. L., Balows, A., Chipps, H. D., and Holloway, J. B., Jr.: Isolated Pulmonary Cryptococcosis, A.M.A. Arch. Int. Med. **99**:285-289, 1957.
201. White, M., and Arany, L. S.: Resection in Pulmonary Cryptococcosis (Torulosis), J. Thoracic Surg. **35**:402-410, 1958.
202. McConchie, I. H., and Hayward, J. I.: Torula histolytica (Cryptococcus neoformans) Granuloma of the Lung Treated With Pulmonary Resection, Postgrad. M.J. **34**:190-194, 1958.
203. Holm, P.: Studies on the Aetiology of Human Actinomycosis. II. Do the "Other Microbes" of Actinomycosis Possess Virulence? Acta path. et microb. scandinav. **28**:391-406, 1951.
204. Glahn, M.: Cervico-facial Actinomycosis—Etiology and Diagnosis. The Pathogenesis of Cervico-facial Actinomycosis, Acta chir. scandinav., **108**:183-192, 1954.
205. Lentze, F.: Zur Frage einer komplexen Aetiologie der Aktinomykose und ihrer Bedeutung

fuer die Therapie, Ärtzl. Forsch. **12**:I/205-208, 1958.

206. Lorenz, O.: Neue Erkentnisse zur Pathogenese und Klinik der cervicofacialen Aktinomykose, Med. Klin. **54**:9-13, 1959.
207. Strauss, R. E., Kligman, A. M., and Pillsbury, D. M.: The Chemotherapy of Actinomycosis and Nocardiosis, Am. Rev. Tuberc. **63**:441-448, 1951.
208. Suter, L. S., and Vaughan, B. F.: The Effect of Antibacterial Agents on the Growth of Actinomyces bovis, Antibiotics **5**:557-560, 1955.
209. Nichols, D. R., and Herrell, W. E.: Penicillin in the Treatment of Actinomycosis, J. Lab. & Clin. Med. **33**:521-525, 1948.
210. Putman, A. C., Dockerty, M. B., and Waugh, J. M.: Abdominal Actinomycosis. An Analysis of 122 Cases, Surgery **28**:781-800, 1950.
211. Wilenius, R.: Actinomycosis With Special Reference to Its Treatment With Penicillin, Ann. chir. et gynaec. Fenniae, suppl. **42**:1-33, 1953.
212. Lesney, T. A., and Traeger, K. A.: Cervicofacial Actinomycosis; a Postextraction Complication, J. Oral Surg. **17**:51-59, 1959.
213. Garrod, L. P.: The Sensitivity of Actinomyces israeli to Antibiotics, Brit. M.J. **1**:1263-1264, 1952.
214. Adamski, J., and Dobek, M.: The Sensitivity of Strains of Actinomyces Wolff-Israeli to Some Antibiotics in In Vitro Tests, Bull. Soc. Amis Sc. Poznan, Ser. C., No. 5, 1955, pp. 3-100.
215. Hanf, U., Heinrich, S., and Legler, F.: Zur Frage der Antibiotica-Resistenz des Erregers der Aktinomykose, Med. Klin. **49**:250-251, 1954.
216. Halde, C., and Newstrand, D.: The Sensitivity of Pathogenic Actinomycetes to Various Sulfonamides and Sulfone Compounds. In Sternberg, T. H., and Newcomer, V. D., editors: Therapy of Fungus Diseases, Boston, 1955, Little, Brown & Company, pp. 147-153.
217. Vanbreuseghem, R., Courtois, C., Thys, A., and Doupagne, P.: Deux cas de mycétomes congolais par Nocardia brasiliensis, Ann. Soc. belge méd. trop. **36**:479-485, 1956.
218. MacKinnon, J. E., and Artagaveytia-Allende, R. C.: The Main Species of Pathogenic Aerobic Actinomycetes Causing Mycetomas, Tr. Roy. Soc. Trop. Med. & Hyg. **50**:31-40, 1956.
219. Derom, E., Francois, J., De Busscher, J., and Hoffmann, G. R.: Entreprise Crâniocérébrale à Nocardia, Acta chir. belg. **57**:463-466, 1958.
220. Basserman, F. J.: Die Nocardiose, Tuberculosearzt **10**:169-175, 1956.
221. Gram, H., and Oehmichen, W.: Nocardiose, Zentralbl. allg. Path. **95**:17-22, 1956.
222. Runyon, E. H.: Nocardia asteroides: Studies of Its Pathogenicity and Drug Sensitivities, J. Lab. & Clin. Med. **37**:713-720, 1951.
223. Rivera, J. V., and Perez, J. B.: Pulmonary Nocardiosis Treated With Chloramphenicol, A.M.A. Arch. Int. Med. **100**:152-156, 1957.
224. MacKinnon, J. E., Artagaveytia-Allende, R. C., and Garcia-Zarron, N.: The Inhibitory Effect of Chemotherapeutic Agents on the Growth of the Causal Organisms of Exogenous Mycetomas and Nocardiosis, Tr. Roy. Soc. Trop. Med. & Hyg. **52**:78-86, 1958.
225. González Ochoa, A.: Effectiveness of DDS in the Treatment of Chromoblastomycosis and of Mycetoma Caused by Nocardia brasiliensis. In Sternberg, T. H., and Newcomer, V. D., editors: Therapy of Fungus Diseases, Boston, 1955, Little, Brown & Company, pp. 321-327.
226. González Ochoa, A., and Ahumada Padilla, M.: Tratamiento del micetoma actinomicósico por la inyección local de la diaminodifenilsulfona, Rev. Inst. salub. y enferm. trop. **18**:41-44, 1958.
227. Mariat, F.: Action in vitro de la 4,4'-diaminodiphenyl sulfone sur les actinomycètes aérobies pathogènes, Compt. rend. Acad. sc. **244**:3095-3098, 1957.
228. Abbott, P. H.: Mycetoma in the Sudan, Tr. Roy. Soc. Trop. Med. & Hyg. **50**:11-24, 1956.
229. Norden, A.: Sporotrichosis: Clinical and Laboratory Features and a Serologic Study in Experimental Animals and Humans, Acta path. et microbiol. scandinav., suppl. **89**:1-119, 1951.
230. Elson, W. O.: The Antibacterial and Fungistatic Properties of Propamidine, J. Infect. Dis. **76**:193-197, 1945.
231. Harrell, E. R., Bocobo, F. C., and Curtis, A. C.: Sporotrichosis Successfully Treated With Stilbamidine, A.M.A. Arch. Int. Med. **93**:162-167, 1954.
232. Geraci, J. E., Dry, I. J., Ulrich, J. A., Weed, L. A., MacCarty, C. S., and Sayre, G. P.: Experiences With 2-Hydroxystilbamidine in Systemic Sporotrichosis: Report of an Unusual Case, A.M.A. Arch. Int. Med. **96**:478-489, 1955.
233. Lea, W. A., Schuster, D. S., and Harrell, E. R.: Mycological Flora of the Healthy External Auditory Canal: A Study of 120 Human Subjects, J. Invest. Dermat. **31**:137-138, 1958.
234. Seligmann, E.: Virulence Enhancement of Candida albicans by Antibiotics and Cortisone, Proc. Soc. Exper. Biol. & Med. **83**:778-781, 1953.
235. Harrell, E. R., and Thompson, G. R.: Systemic Candidiasis (Moniliasis) Complicating Treatment of Bacterial Endocarditis, With Review of Literature and Report of Apparent Cure of One Case With Parenteral Mycostatin, Ann. Int. Med. **49**:207-215, 1958.
236. Woods, J. W., Manning, I. H., Jr., and Patterson, C. N.: Monilial Infections Complicat-

ing the Therapeutic Use of Antibiotics, J.A.M.A. **145**:207-211, 1951.

237. Gonzáles Ochoa, A.: The Status of Fungus Diseases in Mexico. In Sternberg, T. H., and Newcomer, V. D., editors: Therapy of Fungus Diseases, Boston, 1955, Little, Brown & Company, pp. 66-72.
238. Campbell, C. C., Hodges, E. P., and Hill, G. B.: Therapeutic Effect of Nystatin (Fungicidin) in Mice Experimentally Infected With Histoplasma capsulatum, Antibiotics **4**:406-410, 1954.
239. Donovick, R., Pansy, F. E., Stout, H. A., Stander, H., Weinstein, M. J., and Gold, W.: Some in Vitro Characteristics of Nystatin (Mycostatin). In Sternberg, T. H., and Newcomer, V. D., editors: Therapy of Fungus Diseases, Boston, 1955, Little, Brown & Company, pp. 176-185.
240. Stewart, G. T.: Laboratory and Clinical Studies With Nystatin in Post-antibiotic Mycotic Infections, Brit. M.J. **1**:658-660, 1956.
241. Drouhet, E.: Therapeutic Activity of Nystatin (Mycostatin) in Candida Infections. In Sternberg, T. H., and Newcomer, V. D., editors: Therapy of Fungus Diseases, Boston, 1955, Little, Brown & Company, pp. 211-218.
242. Jennison, R. F., and Stenton, P.: Sensitivity of Candida Strains to Nystatin, J. Clin. Path. **10**:219-221, 1957.
243. Robinson, R. C. V.: Candida albicans Infection of the Skin and Mucous Membranes Treated With Nystatin, Antibiotics Annual, 1955-1956, New York, 1956, Medical Encyclopedia, Inc., pp. 851-855.
244. Cohen, A. C.: Pulmonary Moniliasis, Am. J. M. Sc. **226**:16-23, 1953.
245. Emdin, W., and Finlayson, M. H.: Moniliasis of the Central Nervous System in a Child With Recovery, South African M.J. **28**:868-871, 1954.
246. Neuhauser, I.: Successful Treatment of Intestinal Moniliasis With Fatty Acid Resin Complex, A.M.A. Arch. Int. Med. **93**:53-60, 1954.
247. Metzger, W. I., Wright, L. T., and DiLorenzo, J. C.: Effect of Esters of PABA on Candida and Yeast-like Fungi, J.A.M.A. **155**:352-358, 1954.
248. Barlas, O., and Akyel, M.: A Case of Pulmonary Moniliasis, Brit. M.J. **2**:1394-1396, 1952.
249. Grekin, R. H., Cawley, E. P., and Zheutlin, B.: Generalized Aspergillosis. Report of a Case, Arch. Path. **49**:387-392, 1950.
250. Sidransky, H., and Friedman, L.: The Effect of Cortisone and Antibiotic Agents on Experimental Pulmonary Aspergillosis, Am. J. Path. **35**:169-183, 1959.
251. Torack, R. M.: Fungus Infections Associated With Antibiotics and Steroid Therapy, Am. J. Med. **22**:872-882, 1957.
252. Hinson, K. F. W., Moon, A. J., and Plummer, N. S.: Broncho-pulmonary Aspergillosis. A Review and a Report of Eight New Cases, Thorax **7**:317-333, 1952.
253. Tarlatzis, C. B., Panetsos, A. G., and Dragonas, P. N.: The Effects of Some Antibiotics and Chemical Drugs on the Growth of Aspergillus fumigatus, Am. J. Vet. Res. **18**:214-215, 1957.
254. Riddell, R. W.: Fungous Diseases of Britain, Brit. M.J. **2**:783-786, 1956.
255. Stevenson, J. G., and Reid, J. M.: Bronchopulmonary Aspergillosis, Brit. M.J. **1**:985-986, 1957.
256. Librach, I. M.: Primary Pulmonary Aspergillosis. Report of a Fatal Case, Antibiotic Med. **4**:377-380, 1957.
257. Mangiaracine, A. B., and Liebman, S. D.: Fungus Keratitis (Aspergillus fumigatus), A.M.A. Arch. Ophth. **58**:695-698, 1957.
258. Manning, L. K., and Robertson, L.: A Case of Aspergillosis Treated With Nystatin, Brit. M.J. **1**:345-346, 1959.
259. Williams, D. I., Marten, R. H., and Sarkany, I.: Oral Treatment of Ringworm With Griseofulvin, Lancet **2**:1212-1213, 1958.
260. Blank, H., and Roth, F. J., Jr.: The Treatment of Dermatomycoses With Orally Administered Griseofulvin, A.M.A. Arch. Dermat. **79**:259-266, 1959.
261. Brian, P. W.: Studies on the Biological Activity of Griseofulvin, Ann. Botany **8**:59-77, 1949.
262. Friedman, C., Mishkin, S., and Lubliner, R.: Pulmonary Resection for Aspergillus Abscess of the Lung, Dis. Chest **30**:345-350, 1956.
263. Hausmann, P. F.: Pulmonary Aspergilloma, J. Thoracic Surg. **35**:538-541, 1958.
264. Krasnitz, A.: Bronchopulmonary Aspergillosis, New York J. Med. **57**:3852-3856, 1957.
265. Paltauf, A.: Mycosis mucorina, Virchows Arch. f. path. Anat. **102**:543-564, 1885.
266. Smith, H. W., and Yanagisawa, E.: Rhinomucormycosis. Report of a Fatal Case, New England J. Med. **260**:1007-1012, 1959.
267. Fienberg, R., and Risley, T. S.: Mucormycotic Infection of Arteriosclerotic Thrombus of the Abdominal Aorta. Report of a Case, New England J. Med. **260**:626-629, 1959.
268. Garrett, S. D.: Ecological Groups of Soil Fungi: A Survey of Substrate Relationships, New Phytologist **50**:149-166, 1951.
269. Bauer, H., Ajello, L., Adams, E., and Hernandez, D. U.: Cerebral Mucormycosis: Pathogenesis of the Disease. Description of the Fungus, Rhizopus oryzae, Isolated From a Fatal Case, Am. J. Med. **18**:822-831, 1955.
270. Chick, E. W., Evans, J., and Baker, R. D.: Treatment of Experimental Mucormycosis (Rhizopus oryzae Infection) in Rabbits With Amphotericin B, Antibiotics **8**:394-399, 1958.

Book review

A Symposium on the Evaluation of Drug Toxicity, by Walpole, A. L., and Spinks, A. Boston, 1959, Little Brown & Company. 138 pages, 58 illustrations.

This book makes available material from a timely symposium dealing with the evaluation of drug toxicity which was held in England in 1957. Dr. J. M. McMichael was chairman of the first session, during which five papers were presented. The material covered in this session varied from toxicity problems in clinical medicine to the testing of drugs for toxicity. An interesting paper on the action of chemical substances was given by Dr. E. Weston Hurst, who referred to the work on differential toxicity of chloroform for male mice; the greater susceptibility of female mice to neoarsphenamine; the effect of certain barbiturates, especially those with one short and one long branched side chain, a cyclohexenyl group, or a methylated nitrogen in causing longer sleep in female rats; the sex-linked toxic susceptibility to certain organophosphorus compounds, and the differential toxicity of squill for female rats. A list of substances is given which shows sex-linked differential toxicity.

A section devoted to the determination of the efficacy and safety of new drugs by Dr. J. T. Litchfield brought out some of the factors looked for in determining whether a new chemical is interesting and deserving of further investigation or whether it should be rejected after the preliminary screening procedure.

The section on the testing of drugs for toxicity by Dr. J. M. Barnes is most interesting and points out many of the difficulties which must be borne in mind by anyone working in this complicated field. He explains, for example, that the toxic action of a drug may be direct or may depend on some metabolite of the drug. Although there are of course great individual and species differences in ability to handle an administered drug there are surprising similarities and these seem to be more frequent than the differences. Among the many interesting means by which drug toxicity occurs, the work of Garland on megaloblastic anemia from phenytoin was pointed out as an example of a blood dyscrasia produced by drugs leading to a deficiency in folic acid.

The second session, under the chairmanship of Dr. G. Payling Wright, was primarily concerned with allergic reactions

and toxic action of drugs on the bone marrow and liver. A most stimulating discussion of drug action on subcellular structure was also held during this session. The paper, by Dr. W. Jacobson, and the subsequent discussion on the toxic action of drugs on the bone marrow is most stimulating and should be read by all interested in this subject. The portion dealing with cellular resistance to folic acid antagonists is also noteworthy. The evidence presented, showing the ability of leukemia cells to develop special synthetic activities as a defense of the abnormal cell against folic acid antagonists, makes exciting reading.

The book can be highly recommended as most desirable reading. The material presented is basic and affords an excellent summary of most of the important concepts of drug toxicity.

Dale G. Friend, M.D.

No research is ever quite complete. It is the glory of a good bit of work that it opens the way for still better, and thus rapidly leads to its own eclipse. The object of research is the advancement, not of the investigator, but of knowledge.

FROM "THE SPIRIT OF RESEARCH—A PLEA FOR INDEPENDENCE" BY MERVYN GORDON, LANCET 1:807, JUNE 30, 1945, REPRINTED FROM ST. BARTHOLOMEW'S HOSPITAL JOURNAL FOR JUNE, 1920.